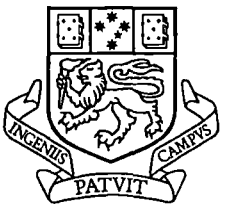


UNIVERSITY OF TASMANIA

ECOTOXICOLOGY OF CONTAMINATED MARINE SEDIMENTS IN TASMANIAN ESTUARIES

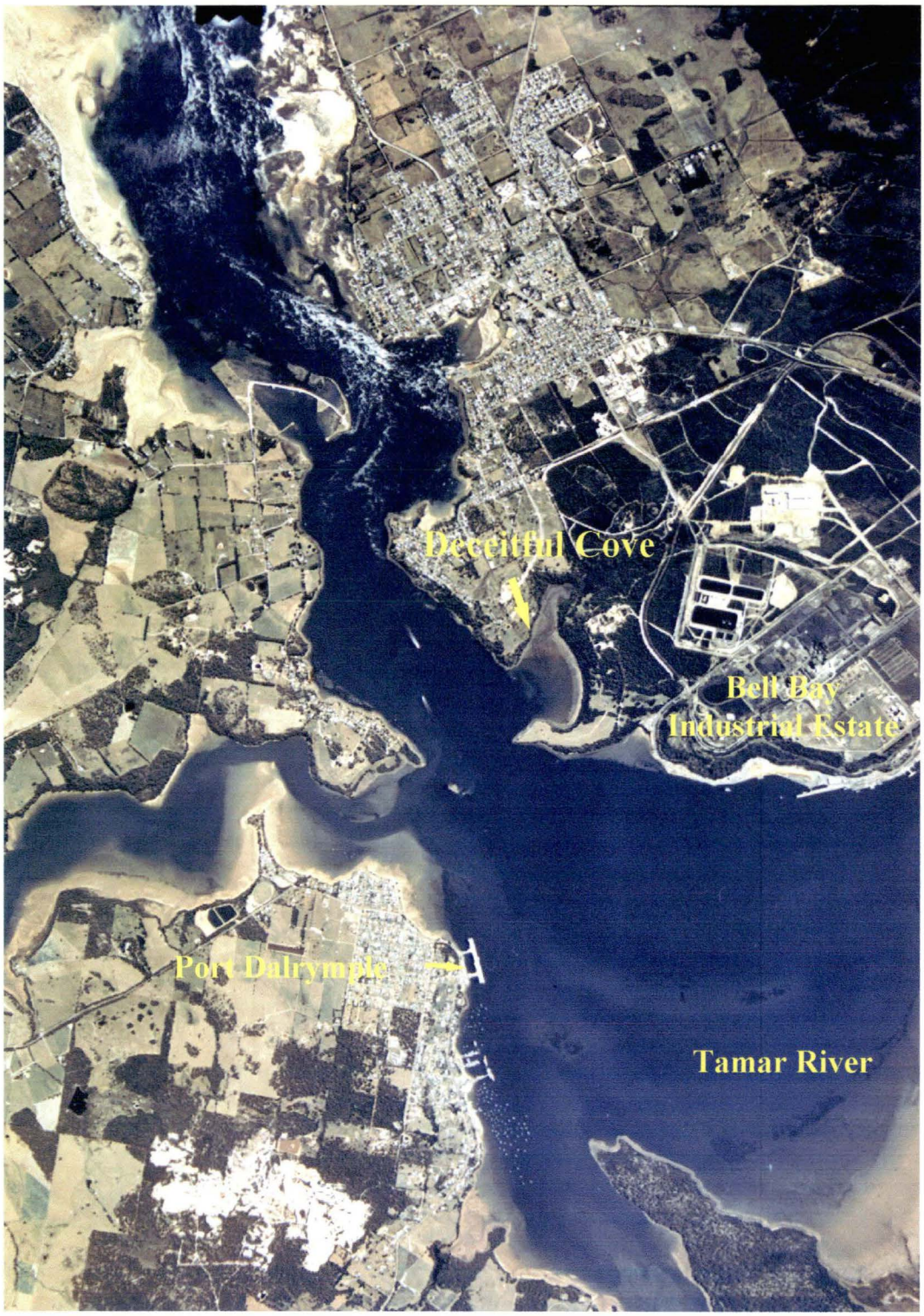
JULIE ANNE MONDON

MA(S.T.S), B.SC.(HONS.), GRAD. DIP. ED.

SUBMITTED IN FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSPHY

FEBRUARY, 2000



Deceitful Cove on the Tamar River estuary. The explorer Captain Matthew Flinders first sighted and named the cove in November 1798.

ABSTRACT

Sediments are components of marine and estuarine ecosystems. Toxicants most often become sorbed to suspended particulate matter, then fall to the bottom to become incorporated into bed sediments. The bioavailability of the toxicants is difficult to measure chemically and the interpretation of biological significance is unclear. Toxicants stored in sediments can be released due to physical and chemical changes in the environment. The extent of the effects of these changes is also not well established and there is little knowledge of what levels of contaminants may be mobilised, and the ecological effect of that mobilisation.

Although recently, pore water toxicity and chemistry has been included in sediment testing, there is a lack of research characterising pore water from Australian marine sediments. Further, there is a paucity of multidisciplinary research of the quality of marine sediments in Australia to provide information on factors affecting quality and remediation of marine sediments that will contribute to improved environmental management of marine sites and allow for successful remediation practices.

The primary aim of this study was to apply a multidisciplinary approach to form a weight of evidence assessment of sediment quality in northern Tasmanian estuaries. The technical objectives associate with this aim were to:

- determine whether shallow subtidal sediments adjacent to a heavy metals industrial estate was chemically contaminated relative to other northern Tasmanian locations, and relative to numerical, effects-based guidelines
- determine if benthic communities exposed to chemical contamination differed relative to benthic communities exposed to non-contaminated sediments
- establish whether the contaminated sediments were toxic relative to non-contaminated sediments
- determine the relationships between toxicity, chemical composition and benthic communities of the subtidal sediments at contaminated and non-contaminated sites
- generate background data for future assessment of sediment remediation.

The relationship between the toxicity, chemical composition and benthic communities was investigated through a field program. Over two years, samples were collected at four locations. One of these, Deceitful Cove in the Tamar River estuary, has been heavily contaminated by past industrial effluents from aluminium refining and ferro-manganese smelting industrial plants, another (East Arm in the Tamar River estuary) has intermediate contamination, while the other two (Squeaking Point and North East Arm in the Port Sorell estuary) are effectively free of pollution. A Sediment Quality Triad (SQT) assessment of shallow subtidal marine sediments within the two adjacent northern Tasmanian estuaries was conducted to assess sediment quality in terms of potential to elicit adverse biological effects. The SQT provides a weight-of-evidence approach integrating toxicological and chemical analysis with benthic community structure to determine likely impacts of contaminants. Generic effects-based sediment quality guideline (SQG) values were also used to facilitate evaluation of sediment quality by identification of potential contaminants or mixtures of contaminants, likely to cause adverse biological effects. Chemical analysis involved assessment of total trace metals and organics concentrations within sediments. Assessment of toxicity involved the adaptation of a suite of first tier screening bioassays, currently used for testing toxicity of marine water: Microtox®, sea urchin larval development inhibition and algal growth inhibition tests. Benthic macroinvertebrate community structure was evaluated using univariate, distributional and multivariate analysis of assemblages: species diversity indices, hierachical cluster analysis and non-metric multi-dimensional scaling ordination.

Differential SQT analysis indicated strong evidence of contaminant-induced stress, and possible environmental degradation in the Tamar River estuary at Deceitful Cove. Additionally, contaminant-induced stress was not restricted to a geographically isolated area adjacent to the industrial estate. Multi-dimensional scaling ordination and univariate analyses identified significant differences between the patterns of distribution and abundance of benthic fauna from contaminated and non-contaminated estuaries. There was a significant correlation between patterns of assemblages and concentrations of trace metals.

The overall findings from the SQT and multivariate analyses strongly suggest that a combination of metal contaminants are directly related to elevated pore water toxicity and alteration in macroinvertebrate community structure. The liquid phase Microtox® and algal growth bioassays are suitable for testing pore water toxicity of Tasmanian coastal marine sediments. However, difficulty in interpreting Microtox® solid phase test results limits the use of this assay for routine testing. Additionally, research on extending the spawning period of the sea urchin *Heliocidaris tuberculata* is necessary before this species can be used for routine bioassay work.

The secondary aims of this project were to determine if toxicity of contaminated sediment at Deceitful Cove could lead to wider ecological impact, and to identify potential biomarkers for assessment and monitoring of effects of pollution on benthic species in the field. The technical objectives associated with these aims were to:

- determine if exposure to contaminated sediments may impact on benthic finfish
- determine the main route of toxicity of sediment bound contaminants
- establish the bioavailability of xenobiotic contaminants in sediments
- establish the ecotoxicological effects of physical disturbance of contaminated sediments on benthic finfish
- generate background data for future assessment of sediment remediation.

Selected biomarkers in benthic finfish were investigated as indicators of anthropomorphic contaminant exposure. Hatchery reared greenback flounder *Rhombosolea tapirina* were exposed under laboratory conditions to reference sediment, and contaminated sediment and contaminated diet collected from Deceitful Cove. Laboratory exposure modelling pulsed resuspension of contaminated sediment was also conducted. Cytochrome P450 induction, using the ethoxyresorufin-o-deethylase (EROD) assay, was elevated in flounder exposed to contaminated sediment and diet. The innate immune response, measured by phagocytic capacity, indicated a reduction in phagocytic efficiency with exposure to contaminated sediment and contaminated diet, whereas measurement of lysozyme response indicated a

stimulatory response when exposed to resuspended contaminated sediment. Exposure to contaminated sediment and diet elicited multi-organ histological alteration: principally partial and total epidermal erosion, and multifocal necrosis of the liver. Growth reduction was evident in flounder exposed to contaminated sediment and diet.

The overall findings from the biomarker research indicate strong evidence to suggest contaminants in Deceitful Cove are immunotoxic and cytotoxic to benthic finish. Contaminant exposure is primarily via direct contact with contaminated sediment and diet. Organics, specifically CYP 1 A-inducing contaminants are present and bioavailable in Deceitful Cove. The histological alteration of the skin in flounder indicates a potentially significant impact on benthic teleost species inhabiting areas of contaminated sediments. Moreover, significant impairment of growth indicates possible reduced survival potential of wild flounder. Additional studies are required to further evaluate the responses of *R.tapirina* identified in this study as potentially effective biomarkers for assessment and monitoring of effects of marine pollution on benthic species in the field.

This is the first published SQT study in Australia, and as with northern hemisphere studies, highlights the limitations associated with relying solely on bulk sediment chemistry or community structure to assess sediment quality. The combination of the SQT incorporating multivariate analysis of community structure, and laboratory-based biomarker assessment, has facilitated the establishment of a sound basis for understanding the nature of the chemical impact from the individual level to the overall ecology of the affected aquatic habits of Deceitful Cove in the lower Tamar River estuary.

The study provides a comprehensive survey of macrobenthic assemblages in two northern Tasmania estuaries with description of environmental conditions as a baseline from which accurate future assessments of environmental changes can be made, and has highlighted several areas of concern where remediation issues may need to be addressed.

ACKNOWLEDGEMENTS

Numerous people have provided great assistance over the course of this project which was jointly funded by the Australian Research Council, TEMCO and Comalco Aluminium (Bell Bay) Limited.

First and foremost, I would like to express my sincere thanks to Dr Barbara Nowak for her invaluable guidance, friendship and encouragement to study in the field of ecotoxicology, and to Professor Alastair Richardson for his constructive criticism and unwavering support. Mr David Hassell, (TEMCO), has wholeheartedly supported this project and expressed keen interest in all stages of its development from start to finish. Dr Bill Woods, Mr Leon Payne and Mr Tim Johns (Comalco) have also contributed significantly during the course of the study.

I would like to acknowledge the generous support of Professor Anders Södergren (University of Lund, Sweden), who was instrumental in facilitating the analysis of organochlorines in tissue and sediment samples, and Dr Graham Edgar (University of Tasmania) and Dr Brian Smith (Queen Victoria Museum) for assistance with macroinvertebrate identification.

Several brave souls, notably Mr Jon Bryan, Ms Ruth Eriksen, Ms Susan Duda, Ms Pollyanna Hilder, Mr Mark Hilder, Ms Marianne Helders, Mr Rob Gurney, Ms Nicole Olland (Holland) and Ms Antje Heinrich (Germany), volunteered their skills and time in the field, sometimes under very trying conditions, and without whom collection of sediment samples, fish and sea urchins would have been extremely difficult.

Great assistance was provided by Ms Ruth Eriksen, Ms Susan Duda, Ms Carmel Pollino, Dr Jill Woodworth , Ms Nicole Olland, Ms Jacqui Winterford, Ms Pollyanna Hilder and Mr Mark Hilder, by way of technical assistance with selected assays, histological slide preparation, and fish husbandry, and by Dr Natalie Moltschaniwskyj for advice on statistical analysis.

Besides those already mentioned, 1 would very much like to thank Professor Ned Pankhurst, Dr Tish Pankhurst, Dr Barry Munday, Dr Danielle Johnson, Dr Chris Burke, Dr Peter Thompson and the other members of the academic, technical and clerical staff at the School of Aquaculture, who have all offered invaluable advice and encouragement over the course of this study.

Additionally, some of the greatest help has come from fellow postgraduate students and post doctoral fellows at the School of Aquaculture (1996-2000), and whose friendship and goodwill I deeply appreciate.

Finally, I must thank my family and friends for enduring my many years of study and not visiting home as often as I should have, and especially Del, whose patience and understanding of my desire to sit entombed in the study in front of a computer screen for hours on end, also deserves an award.

This thesis is dedicated to the memory of my grandfather, Geoffrey, who unexpectedly passed away six months before the project was completed. His passion for science and natural history has been, and will continue to be, a great inspiration.

DECLARATION

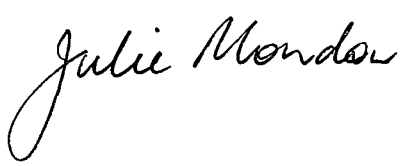
This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text.

JULIE ANNE MONDON

University of Tasmania
February 2000

AUTHORITY OF ACCESS

This thesis may be made available for loan and limited copying in accordance with the Copyright Act, 1968.



JULIE ANNE MONDON

University of Tasmania
February 2000

CONTENTS

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Appendix 1

Appendix 2

Appendix 3

Appendix 5

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Appendix 1

Appendix 2

Appendix 5

ACRONYMS

ANOVA	analysis of variance
AVS	acid volatile sulfide
BACI	before-after / control-impact
CSIRO	Commonwealth Scientific and Industrial Research Organisation (Australia)
DDT	dichlorodiphenyltrichloroethane
EC	effect concentration
ERA	ecological risk assessment
ERL	effects range low
ERM	effects range median
EROD	7-ethoxyresorufin 0-deethylase
GC-MS	gas chromatography - mass spectrum
GPS	Global Position System
HPAH	high molecular weight PAH
ICP-MS	inductively coupled mass spectrometry
LOEC	lowest observed concentration
LPAH	low molecular weight PAH
MFO	mixed function oxidases
NOEC	no-observed-effects concentration
NOEL	no-observed-effects level
PAH	polycyclic aromatic hydrocarbon
PCA	principle components analysis
PCB	polychlorinated biphenyl
PEL	probable effect level
SERA	sediment environmental risk assessment
SQG	sediment quality guideline
SQT	sediment quality triad
SQV	sediment quality values
TIE	toxicity identification evaluation
TOC	total organic carbon
UV	ultraviolet

CHAPTER ONE

General Introduction

Estuaries lie at the interface between marine, freshwater, terrestrial and atmospheric systems, and form an integral part of the coastal zone. They comprise some of the most dynamic ecosystems on earth (Edgar *et al.*, 1999), so that much of the flora, fauna and habitats of estuaries are of great scientific, aesthetic and commercial interest.

The quality of coastal water quality is inextricably linked to inputs from the land, and estuaries and their catchments are the main conduits for contaminants entering the coastal zone. Estuaries naturally protect the quality of coastal waters by diluting, filtering and settling out sediments and excess nutrients (Tagaza, 1995). Pollutants from point sources such as industrial and sewage treatment plants add to the nutrients, trace metals, organochlorine and hydrocarbon run-off from urban and agricultural centres, with the inevitable result that estuarine waters are the most heavily affected by pollution (Tagaza, 1995; Edgar *et al.*, 1999).

Sediments are the ultimate repository for most of these anthropogenic contaminants entering the waterways. Contaminants are continually exchanged between the water column and bottom sediments (Forstner, 1987) where they accumulate for months or years, to be released slowly even though the source of the contamination may have been removed (Luoma, 1990). The bioavailability of trace metals and organic contaminants in sediments and porewaters is difficult to measure chemically, and the interpretation of biological significance of chemical analysis is uncertain (Phillips, 1980; Waldichuk, 1985; Chapman, 1990; Phillips & Rainbow, 1993; Matthiessen *et al.*, 1995). The development and field validation of sediment quality guidelines (SQGs), however, has improved the ability to predict toxicity in recent years (Long *et al.*, 1995). Uses of SQGs as aids in the interpretation of such data can help form a weight of evidence in sediment toxicity studies.

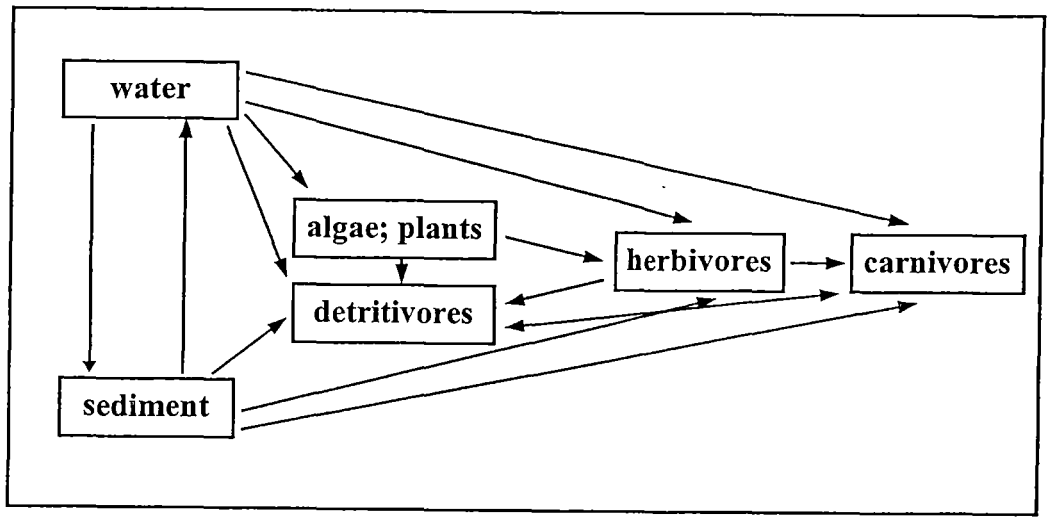


Figure 1. Metal pathways to benthic organisms (modified from Chapman *et al.*, 1998).

Sediment is a complex environment in which chemicals partition between sediment particles, porewater, overlying water and benthic biota (Figure 1). Partitioning of contaminants between sediment and water, and successive trophic levels, results in the potential for the bioavailable fraction of the contaminant to be absorbed by the skin, embryological or respiratory membranes of the organism, or via the digestive system (Courtney, 1980; Burton, 1992; Peters *et al.*, 1996). Interactions between the bioavailable contaminants and endogenous molecules of the organism can vary depending on the neutrality or activity of the contaminants taken up. The neutral fraction, ie. the bioavailable compounds that do not produce a direct toxic response, may bioaccumulate within the organism (Figure 2). Alternatively, the neutral fraction may elicit biotransformation and excretion processes (detoxification), potentially producing an indirect toxic response, as in the case of certain organochlorines (Stegeman & Lech, 1991). Specific interactions associated with the active fraction of the contaminant load may result in direct toxic effects at the biochemical level, and in the worst case produce altered individual responses that can affect the population, community, and ultimately the ecosystem level (Figure 2). Thus, the biological response to contaminants can occur at various levels of biological organisation and extend over considerable periods of time.

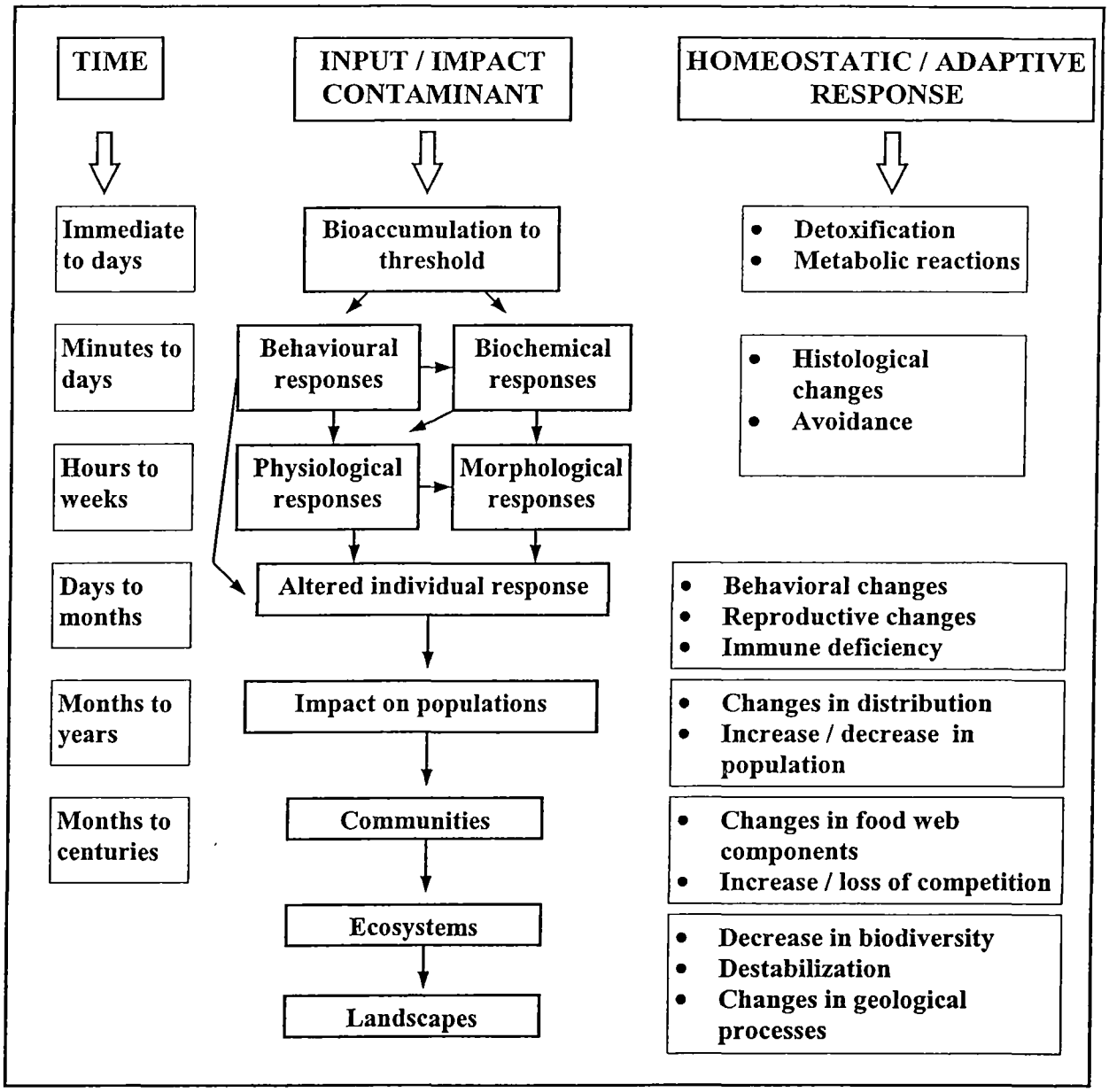


Figure 2. Generalised scheme of contaminant impact at various levels of biological organisation (*Anon.*)

Reliance on chemical analysis alone as an indicator of aquatic health fails to recognise the complexity of toxicant effects within the biological system described above. Responses may be elicited at chemical concentrations below analytical detection limits, or from sediments after direct chemical exposure has ceased (Matthiessen *et al.*, 1995). Different taxa also exhibit varying susceptibilities to the contaminants present in contaminated marine and estuarine habitats (Matthiessen *et al.*, 1998). The ecological significance of levels of trace metal contamination, for example, hinges on the uptake and sequestration of contaminants by organisms in their ambient environment. Despite the role of several trace metals (Cr, Mn, Fe, Ni, Cu, Zn and Al) in essential physiological functions (Simkiss & Taylor, 1989), any metals taken up by organisms may be toxic; however, their precise toxicity varies with speciation of the metal (Phillips, 1993). Such factors preclude the accurate prediction of environmental impacts from chemical data alone. Consequently, marine environmental assessment and monitoring must necessarily include the measurement of biological effects (Matthiessen *et al.*, 1995), covering several taxonomic groups (Matthiessen *et al.*, 1998), and if possible, several levels of biological organisation including effects at the community and ecosystem levels (Maund *et al.*, 1999).

A comprehensive understanding of the environmental impact of pollutants in benthic marine ecosystems therefore requires a holistic, multidisciplinary approach that is relatively uncommon in traditional science. No universally accepted method for sediment quality assessment exists. Several approaches have been used including reference area comparisons, tissue residues, equilibrium partitioning, interstitial water toxicity, whole sediment toxicity and sediment spiking tests, and benthic community structure (Ingersoll, 1995). Whereas each approach provides valuable information relating the chemistry to the particular endpoint of the method applied, an integration of several methods using a weight of evidence is needed to assess the effects of contaminants associated with sediment (Ingersoll, 1995). The Sediment Quality Triad (SQT) (Long & Chapman, 1985) is a multi-disciplinary alternative to using chemical measurements as surrogate indicators sediment quality. This approach characterises the contamination of sediments in aquatic environments, and the impacts deriving from the contamination, by employing

simultaneous chemical analysis, toxicity testing and evaluation of benthic community structure (Chapman, 1990). Conclusions relating to the ecological impact of sediment associated contaminants are inference based, using multiple lines of evidence (Long & Chapman, 1985). Regarded as a powerful integrated screening tool for assessing existing impacts on sediment communities (Ingersoll *et al.*, 1997), the SQT approach is valuable where *a priori* environmental data is lacking or non-existent.

Deceitful Cove, on the lower reaches of the Tamar River estuary in northern Tasmania, is one such area where considerable contamination from smelting and metal based industries has occurred (Gawne & Richardson, 1992; Miedecke, 1992a). In response to a paucity of baseline data evaluating the ecological impact of this contamination, a short-term preliminary field survey was conducted (Mondon, 1996, Appendix 1). Chemistry, toxicity and ecology of shallow subtidal sediments of Deceitful Cove, were compared to sediments from putatively less contaminated and non-contaminated sites. The results suggested a possible link between chemical contamination of porewater and elevated toxicity, warranting a full SQT assessment to confirm the character of the relationships between the sediment chemistry, toxicity and benthic assemblages of contaminated sites in the lower Tamar River estuary.

Because of their intimate contact with the benthos, all benthic species are potentially susceptible to sediment associated contaminants. Whilst a SQT assessment of Deceitful Cove sediments would provide a sound predictive assessment of the state of the benthic environment at the time of survey, the ecological relevance and predictive ability of laboratory tests are often uncertain, thus requiring *in situ* biological analysis. The potential variability in contaminant response between different levels of trophic organisms emphasises the need for a multi-assay approach to pollution evaluation that extends beyond unicellular test organisms to include vertebrates and longer-term chemical exposures, particularly given the short duration, single pathway chemical exposure associated with unicellular bioassays (Matthiessen *et al.*, 1998).

A further consideration concerning evaluation of sediment quality relates to the physical stability of the substrata. Disturbance to sediments through natural occurrences such as burrowing and storm activity, or anthropogenic measures such as dredging, increases the potential bioavailability of previously sediment-bound contaminants (Forstner, 1987; Ciarelli *et al.*, 1998). Bioconcentration of contaminants released from dredged sediments is also known to occur (Seelye *et al.*, 1982), and presents a justifiable concern for management of contaminated sediments in the Tamar River estuary. A remediation proposal to dredge Deceitful Cove is currently under consideration (D. Hassell, *pers. comm.*), and although it is well known that dredging overturns large volumes of sediments, there is no local knowledge regarding the ecological impact of contaminants mobilised from sediment disturbance.

With this background, the technical objectives of the project are:

- to establish the sediment contamination levels at Deceitful Cove relative to other sites in northern Tasmania and relative to numerical, effects-based guidelines
- to determine if benthic macroinvertebrate communities at Deceitful Cove differ from other sites in northern Tasmania
- to establish whether Deceitful Cove sediments are toxic relative other sites in northern Tasmania
- to determine the relationships between toxicity, chemical composition and benthic macroinvertebrate communities of the subtidal sediments at contaminated and non-contaminated sites in northern Tasmania
- to determine if exposure to contaminated Deceitful Cove sediments may impact on benthic finfish
- to establish the main route of toxicity of Deceitful Cove sediments for finfish
- to establish the bioavailability of xenobiotic contaminants present in Deceitful Cove sediments
- to establish the ecotoxicological effects of physical disturbance of Deceitful Cove sediments on benthic finfish

- to identify potential biomarkers for assessment and monitoring the effects of marine pollution on benthic finfish in the field
- to generate background data for future assessment of sediment remediation.

The research design involved two investigative paths. The first included an assessment of sediment quality using the Sediment Quality Triad (SQT) to determine the likelihood of deleterious environmental impacts having occurred, or the likelihood of that occurrence as a result of toxic chemical exposure. Sediment quality guidelines (SQGs) were used to establish the probabilities of toxicity based upon the chemical data. The actual occurrence of toxicity was established with a suite of laboratory tests (Figure 3). A detailed investigation of the macroinvertebrate community assemblages was conducted to establish whether certain species were characteristic of assemblages in contaminated and reference locations, and to determine the contaminant(s) most likely responsible for those differences.

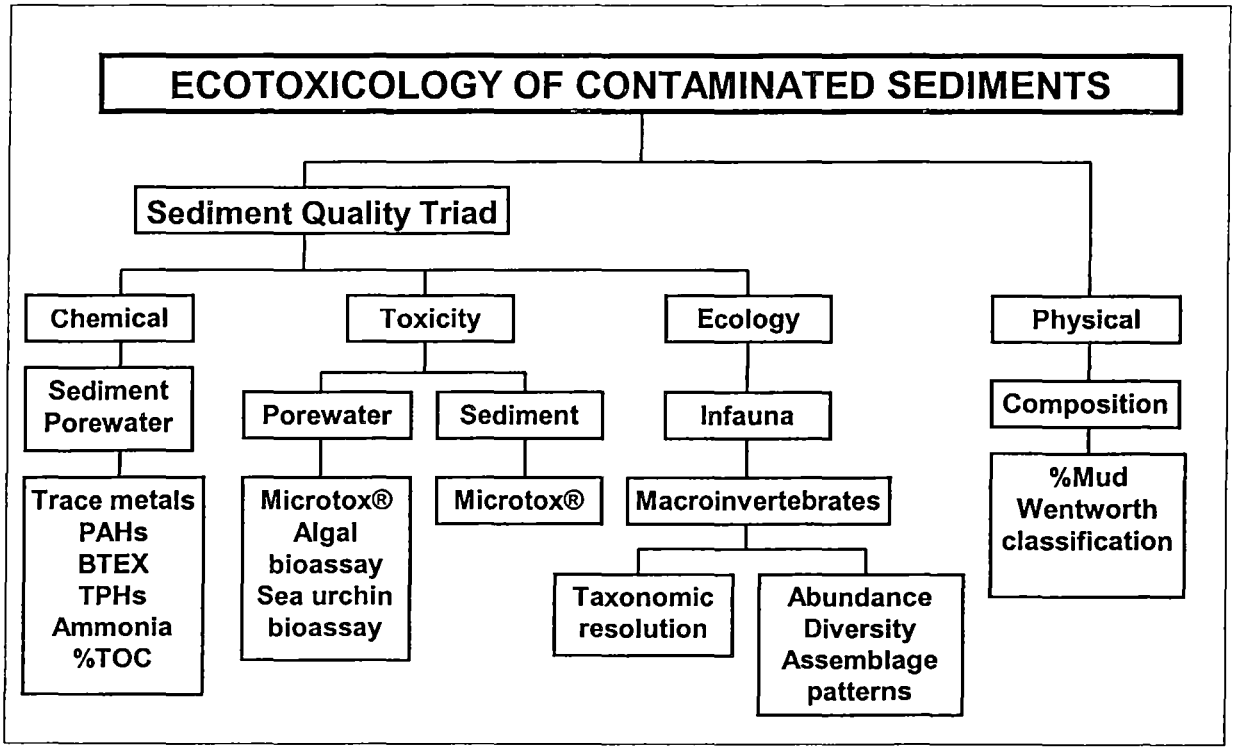


Figure 3. Sediment Quality Triad research plan and methodology.

The second line of investigation involved analysis of selected biomarkers in benthic finfish as indicators of contaminant exposure (Figure 4). Multiple exposure routes were investigated: sediment, overlying water and diet, in addition to evaluating the impact of sustained sediment disturbance on a local benthic finfish, the greenback flounder *Rhombosolea tapirina*. Biomarker responses were assessed at the biochemical, physiological, morphological and individual levels.

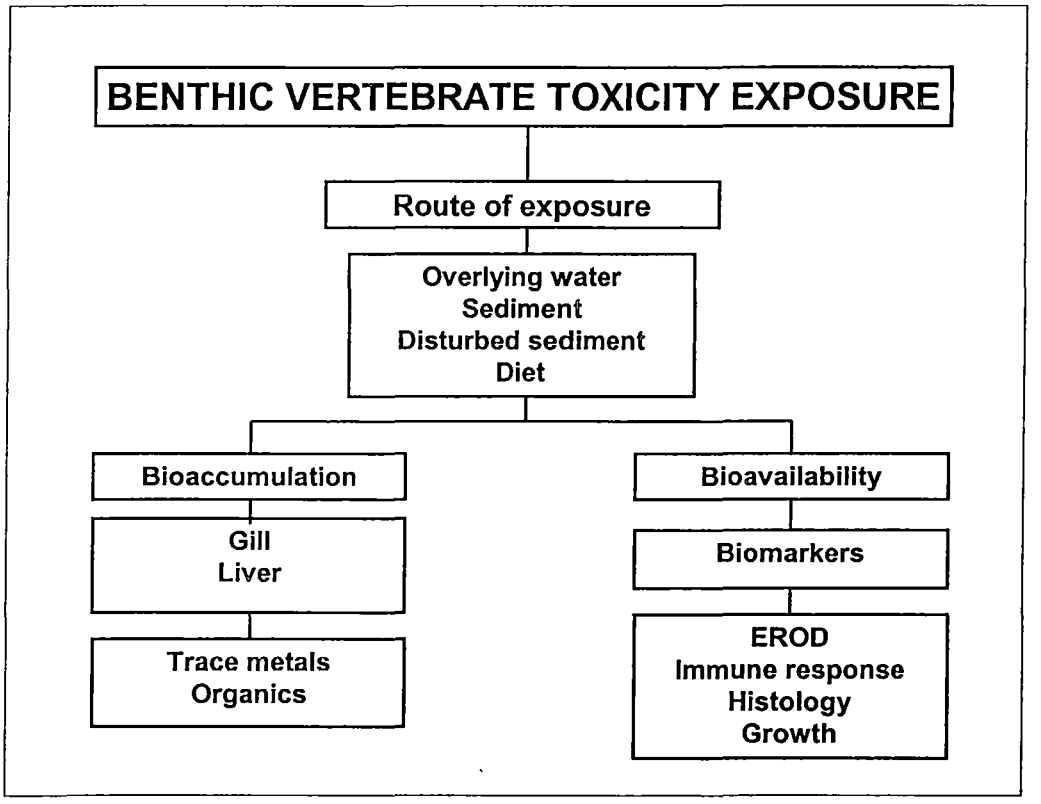


Figure 4. Vertebrate biomarker reseach plan and methodology.

The following chapters present the major findings of the study as a series of manuscripts submitted for publication. The current status of each manuscript is presented at the beginning of the relevant chapter. Unless otherwise stated, the manuscripts are presented as submitted, without alteration. However, the general formatting of each chapter has been kept uniform. Chapter 2 outlines the Sediment Quality Triad (SQT) analysis of the shallow

subtidal marine sediments in two northern Tasmanian estuaries. Sediment quality of Deceitful Cove is assessed in conjunction with less contaminated and reference sediments, to determine the likely degree of stress or degradation associated with elevated chemical concentrations in the aquatic environment. Chapter 3 explores the benthic assemblage patterns in detail, with an emphasis on identifying discriminating species and linking measured environmental variables directly to assemblage patterns. Chapters 4 and 5 present two manuscripts relating to the biomarker assessment of the greenback flounder *Rhombosolea tapirina.* Chapter 4 discusses the histological, growth and 7- ethoxyresorufin 0-deethylase (EROD) activity responses of greenback flounder to contaminated marine sediment (disturbed and undisturbed), and contaminated diet. The second paper, Chapter 5, concentrates on the non-specific immune response of greenback flounder to long-term contaminated marine sediment (disturbed and undisturbed), and contaminated diet.

Other studies directly associated with the overall project (the 1995/1996 preliminary field survey, effects of storage temperature and time on toxicity of contaminated sediment and porewater samples, and the taxonomic resolution of benthic infauna required to identify benthic community response to anthropogenic disturbances) are included in the Appendix. A separate study of organochlorine contamination of sediments and biota in northern Tasmanian estuaries and coastal waters is also included.

CHAPTER TWO

Sediment Quality Triad assessment of trace metals and organic contamination of shallow subtidal marine sediments near a ferro-manganese smelter and aluminium refining plant

J.A. Mondon[1], A.M.M. Richardson[2] and B.F. Nowak[1]

[1]School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Launceston, Tasmania 7250, Australia
[2]School of Zoology, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Sandy Bay, Tasmania 7000, Australia

Submitted for publication in *Environmental Toxicology & Chemistry*

ABSTRACT

Trace metal uptake by living organisms is partly controlled by concentrations of trace metals dissolved in sediment porewaters. Uptake of these elements and synthetic compounds may be acutely toxic to some organisms, conceivably causing local extinctions. Over a period of two decades discharge of wastes primarily from aluminium refining and ferro-manganese smelting industrial plants, concentrated trace metal toxicants within subtidal sediments of Deceitful Cove, Tamar River, Tasmania. A sediment quality triad (SQT) assessment of shallow subtidal marine sediments in two adjacent northern Tasmanian estuaries was conducted to assess sediment quality in terms of its potential to elicit adverse biological effects. Application of the SQT has largely been confined to northern America and has not been applied to Australian estuaries or coastal waters. Generic effects-based sediment quality guideline (SQG) values were also used to facilitate evaluation of sediment quality by identification of potential contaminants, or mixtures of contaminants, that are likely to cause adverse biological effects. Differential SQT analysis indicated evidence of contaminant-induced degradation at Deceitful Cove. Contingency table

analysis strongly suggests that contaminant loading influences the benthic fauna assemblage due to toxicity associated with the chemicals present. SQGs and principle components analysis (PCA) also suggest that multiple metal and organic contaminants are directly related to elevated pore water toxicity and decreased species diversity. This study has generated baseline data for future assessments of changes in sediment quality, and has highlighted several areas of concern where remediation issues need to be addressed.

Introduction

Sediment is widely regarded as a repository for many toxicants in the aquatic environment and can serve as a sink and source for contaminants through the processes of deposition, diffusion, adsorption, oxidation, complexation, resuspension and emigration (Seelye *et al.*, 1982; Bierl & Umlauf, 1987; Chapman, 1990; Phillips, 1993; Ingersoll *et al.*, 1997). Persistent toxicants such as trace metals and organic chemicals are sorbed to sediments, and contaminant exchange between the sediments and water column has the potential to affect marine biota of all phyla (Seelye *et al.*, 1982; Phillips, 1993). Uptake of dissolved trace metals in pore water and organic compounds may be acutely toxic to some organisms and may bioaccumulate, effectively dispersing the impact range of the contaminant away from its original deposition site (Luoma, 1990).

When an association between chemical contamination and biological effects is suspected, determining cause-effect relationships resulting from chemical contamination of sediment is often problematic (Chapman, 1990). Bioavailability of chemicals is difficult to measure chemically and the interpretation of biological significance of chemical analysis is generally unclear (Chapman, 1990; Matthiessen *et al.*, 1995). Further, toxicological responses to contaminant exposure are frequently generic indicators of stress and cannot be used to discriminate specific factors eliciting the response (Ingersoll *et al.*, 1997). The sediment quality triad (SQT) assessment (Long & Chapman, 1985), provides a weight-of-evidence approach that integrates toxicity assays with field measurements, whereby toxicological and chemical analysis is linked with benthic community structure to determine likely effects (Chapman *et al.*, 1987). Generic effects-based guideline values, such as sediment quality guidelines (SQGs) developed by Long *et al.* (1995) and MacDonald *et al.* (1996), also facilitate evaluation of sediment quality by identification of potential contaminants, or mixtures of contaminants, that are likely to cause adverse biological effects. Sediment quality guidelines are based on an effects database and weight-of-evidence approach for a range of chemicals and chemical concentrations associated with known adverse biological effects within the field and laboratory. MacDonald *et al.'s* (1996) threshold-effects level (TEL) and probable-effects level (PEL) concentrations

indicate environmental chemical concentrations which are rarely (TEL) and frequently (PEL) associated with adverse biological effects. Effects range low (ER-L) and effects range median (ER-M) values (equivalent to the 10th and 50th percentile of effects data respectively) (Long *et al.*, 1995) predict the concentrations above which adverse biological effects are likely to occur. Sediment quality guidelines, used extensively in a variety of sediment assessment applications, have been demonstrated to be accurate measures for predicting toxic and non-toxic responses in the field (Ingersoll *et al.*, 1997), particularly when applied to fine-grained sediment (Long *et al.*, 1998). Additionally, SQG values are typically conservative, minimising the likelihood of false negatives during ecological risk assessment of contaminated sediments (Chapman *et al.*, 1997). Further analysis of sediment chemistry, toxicity and benthic fauna data is possible using the differential analysis approach (Chapman, 1990), which facilitates direct comparison between a putatively impacted area with relatively non-contaminated reference sites thought to support similar habitats, faunal assemblages and geochemical processes.

Although the SQT approach has largely been confined to northern America (Canfield *et al.*, 1994; Pascoe *et al.*, 1994; Besser *et al.* 1996; Canfield *et al.*, 1996; Carr *et al.*, 1996; Chapman *et al.*, 1996; Paine *et al.*, 1996; Canfield *et al.* 1998;, Wildhaber & Schmitt, 1998), with limited application elsewhere (Delvalls & Chapman, 1998), in conjunction with SQGs, use of the SQT as an initial screening system is appropriate to evaluate the pollution status of sediments in any region where little or no ecotoxicological assessment has been made. Tasmanian coastal marine and estuarine waters have received little attention with respect to comprehensive integrated chemical, toxicological and ecological studies. The Tamar River, for example, is one of Tasmania's largest estuaries, supporting commercial and recreational fisheries, marine fish aquaculture and extensive native and migratory water bird populations. Much of the river is classified as a wildlife sanctuary and listed on the Register of the National Estate (Tamar River Master Planning Authority Report, 1990). Recent industrial development has favoured sites and facilities in the lower reaches of the river where the largest area of shallow marine / semi-estuarine habitats suitable for fish nurseries is found (Tamar River Master Planning Authority Report, 1990). During the past 26 years few studies of anthropomorphic contamination have been undertaken in the

estuary (Thrower & Eustace, 1973). Historically the lower reaches have received urban run-off, untreated sewage discharges and mining effluent. Additionally, industrial activity has contributed to trace metal and organic contaminant loading within the region. Deceitful Cove, situated in the lower reaches of the Tamar River, has received drainage from the Bell Bay industrial estate, which includes an aluminium refining plant and a ferro-manganese smelting operation. For twenty to twenty-five years, untreated industrial wastes were released into Deceitful Cove via a storm water outlet and it is possible the bay has been environmentally degraded. Within the last ten years, direct release of waste water has ceased in favour of treated waste water discharge via separate deep water outlets. However, the storm water drain entering the cove receives periodic run-off from the industrial estate after rain and occasional storm water effluent overflows from one of the major industries. The relative importance of historical mass input of chemicals into Deceitful Cove sediment and the Port Dalrymple region is not known.

The aims of this study were two-fold: first, to assess the sediment quality of Deceitful Cove in relation to other putatively contaminated and non-contaminated sites within the lower reaches of the Tamar River Estuary and the adjacent and relatively undisturbed Port Sorell estuary; second, to generate baseline data from which to monitor change in sediment quality in the future. A multi-disciplinary field program comprising simultaneous investigation of chemical contamination, toxicity and benthic ecology was conducted over a two year period. The SQT approach was adopted to facilitate a close approximation of the contamination levels that might cause toxicity in the lower Tamar River estuary, and Deceitful Cove in particular, and what effects the toxicity may have on the ecology of the region.

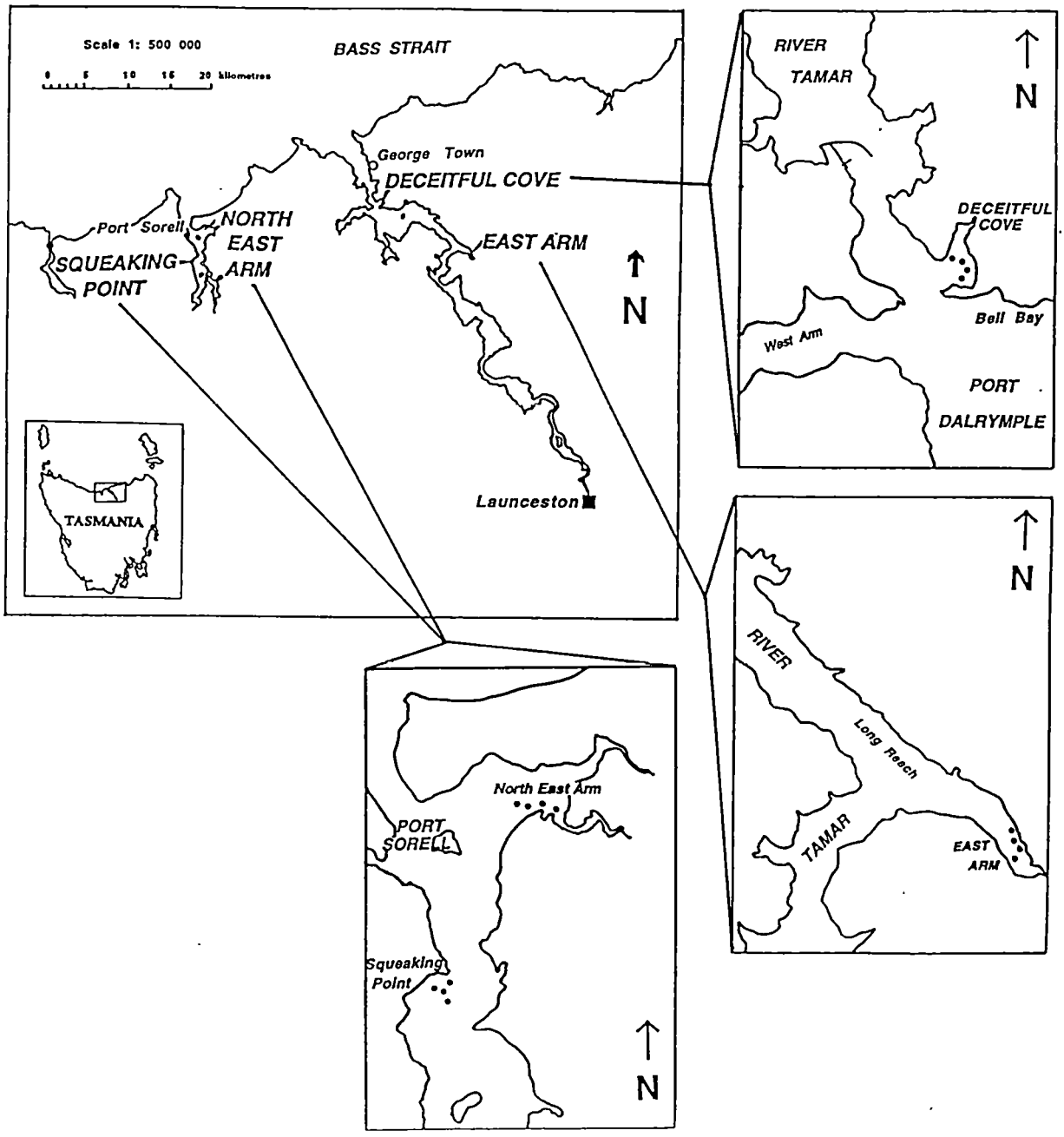


Figure 1. **Location of research area: Deceitful Cove and East Arm in the Tamar River estuary; Squeaking Point and North east Arm in the Port Sorell estuary. Black circles represent sampling sites.**

Methodology

Field survey design

Sediment samples were collected over two years (1996-1997) from four sites within four locations: Deceitful Cove and East Arm in the Tamar River Estuary, and Squeaking Point and Bakers Beach in the Port Sorell Estuary (Figure 1). Sampling was conducted twice during summer and twice during winter, with a minimum two week period between sampling. Sampling locations were based on previously collected data (Mondon, Unpublished): Deceitful Cove sites were selected to represent worst-case conditions in the contaminated region of the Tamar River estuary; intermediately polluted sites at East Arm and reference sites at Port Sorell (Squeaking Point and Bakers Beach) were selected to represent moderate and best-case conditions respectively. In the absence of historical ecotoxicological data for the lower Tamar River estuary, the reference locations were employed as reference sites, with the full recognition that all factors, excluding anthropogenic contaminants, present in the Tamar River estuary, may not be present at Port Sorell. The positions of sites within each location were marked using a series of visual fixes from the shore with the aid of an Eagle AccuNav Sport™ GPS (accuracy 15-100 m). Sampling was conducted at the lowest tide and involved collection of sediments from approximately 0.5 - 1 m depth below surface water. On each occassion replicate samples were collected from four sites within each location for geochemical sediment analysis and classification, contaminant analysis and pore water extraction. Four samples were collected per site and later pooled for benthic macroinvertebrate analysis.

Sediment collection and pore water extraction

Sediment cores were collected using 7 cm diameter polycarbonate benthic corer tubes inserted by hand into the sediment to a depth of 10 cm below the sediment/water interface. Corers were employed to minimise loss of surface sediment due to bow wave generation by benthic grabs and similar devices, and to minimise potential long term gross disturbance of the sediment. Whole sediment cores were placed in sealed, inert, acid-washed containers and kept in an ice-cooled cooler during transportation to a 4°C refrigeration unit. Vacuum extraction of sediment pore water was conducted in the laboratory at 4°C (dark) within 16

hours of collection. Potential contamination from toxic metabolites is known to accumulate in sediments if pore water is extracted later than 24 hours after collection (Carr *et al.*, 1989). Surface water was carefully removed from sediment core samples by siphon and glass pipette. A fused-glass air stone attached with a section of Teflon® tube to a 50 mL acid washed polypropylene or glass syringe (sipper device) was inserted into extracted pooled sediment cores (Winger & Lasier, 1991). The potential for small amounts of contaminants to be adsorbed to the extraction device is considered to be non-significant, (Winger & Lasier, 1991). Extracted pore water was centrifuged to remove particulate matter using an Econospin Sorvall Instruments DuPont centrifuge, for seven munutes at 2500 rpm, then frozen immediately and stored at -20°C, since the toxicity of pore water extracted from sediment and stored at 4°C for longer than 48 hours prior to analysis may alter due to bacterial activity (Carr, 1998).

Granulometry

Particle size of sediment was determined using mechanical dispersion and a modified Bouyoucos hydrometer method. Oven dried (60°C) sieved (2mm Endecott) sediment was soaked in 6% hydrogen peroxide over night to remove organic matter, then boiled with distilled water until active frothing ceased. When cool, calcium carbonate was removed using 2M hydrochloric acid and left to settle. The soil suspension was washed three times with distilled water, allowed to settle and the supernatant siphoned off, then placed on an orbital shaker overnight after the addition of 1M sodium hydroxide and 10% Calgon. The soil suspension was then transferred to a 1000 mL graduated cylinder and shaken vigorously for 2 minutes. Hydrometer readings at 4 minutes 48 seconds post shaking (adjusted for temperature above and below 19.4°C) were divided by sediment weight to calculate the proportion of silt + clay (expressed as a percentage). Readings at 5 hours were used to calculate the proportion of clay. Wentworth classification of sediment based on textural size classes (phi) of oven dried sediment (60°C) was determined using a Rapid Sediment Analyser, developed at the University of Waikato, New Zealand.

%TOC

Total organic carbon was determined by loss on ignition. Sediment samples were oven dried at 80°C, washed with MilliQ water to remove salt, acidified overnight with 2M HCl to remove HCO_3 and dried at 80°C. Crucibles containing 1.5 g dry wt. sediment were burnt in a muffle furnace (650°C) overnight and re-weighed.

Chemical analysis

Sediment for organics analysis was collected as described above, placed in acid washed glass storage containers and preserved frozen prior to transportation and analysis. Polycyclic aromatic hydrocarbons (PAHs) were analysed by Analabs (Hawthorn, Victoria) using gas chromatography - mass spectrum (GC-MS). Sediment samples were mixed with sodium sulphate, and dichloromethane/acetone was then added prior to each sample being sonicated and shaken, and the extract analysed. Sediment for trace metals analysis was oven dried at 100°C overnight, and digested in concentrated HNO_3 for three days after which time the soil/acid mixtures were microwaved for 1-2 hours at a low power setting (low heat) to further the digestion process. The resultant mixture was diluted with distilled water and internal standard added. All samples were filtered prior to ICP-MS (Inductively Coupled Mass Spectrometry , Finnigan - MAT Element) analysis at the Central Science Laboratories, University of Tasmania. Values generated were the mean of 10 scans over each element (1 second dwell time per element).

Potential environmental impact of contaminant levels on resident biota was determined by direct comparison of sediment chemistry data to effects-based sediment quality guidelines (SQGs) developed by Long *et al.* (1995) and MacDonald *et al.* (1996). The strongest evidence of pollution induced degradation was interpreted as those locations showing elevated chemical contamination relative to the reference sites where one or more chemicals exceeded the SQG effects range-median (ER-M) values of Long *et al.* (1995) or the probable effects level (PEL) values of MacDonald *et al.* (1996), and where relatively high mean ER-M quotients (greater than 1.0) existed (Long *et al.* 1998). The SQG quotients, used to determine the degree to which the SQGs were exceeded in concentrations of chemical mixtures, were normalised to SQGs by dividing the concentration of individual

chemicals by their respective ER-Ms (Long *et al.*, 1998). The mean ER-M quotient was calculated as the sum of the quotients for each chemical, divided by the number of chemicals.

Toxicity bioassays

Three bioassays formed a first tier screen for pore water toxicity: algal growth inhibition, sea urchin larval development and Microtox® light emission inhibition. Pore water samples, collected as described above, were frozen immediately after extraction and stored at -20°C. Samples were thawed at 4°C overnight prior to analysis. Toxicity of pore water is not affected by the freezing and thawing process (Carr & Chapman, 1995). All seawater used as diluent for assays was collected from a clean ocean site (Lulworth, Bass Strait), and filtered (0.4 μm) prior to use. Hypersaline brine, if necessary, was prepared by evaporation of natural seawater. Pore water parameters were measured prior to analysis: salinity was determined using a Shibuya Optical S-10 salinity refractometer, un-ionised ammonia (NH_3-N) by salicylate method using a Hach - DR/2000 spectrophotometer, and pH by Activon 209 pH/mV meter.

An algal growth inhibition test, developed by Stauber *et al.* (1994), using the marine diatom *Nitzchia closterium* to measure the chronic toxicity of pore water, was chosen due to its widespread distribution in the Australian temperate coastal zone. A five day old *N. closterium* culture inoculum of $4\text{-}6*10^4$ cells/mL was incubated in replicated 1:2 serial dilutions of pore water containing 0.5 mL of 26mM sodium nitrate and 0.5 mL of 1.3mM potassium dihydrogen phosphate (50 mL final volume) at room temperature, under a 12 hour daylight (14000 lux fluorescent) / 12 hour dark regime, for 72 hours. Final cell density was determined using an Improved Neubauer haemocytometer. The test endpoint, algal growth inhibition, is expressed as a percentage pore water concentration 72 hour EC_{50} algal growth inhibition value. The lower the EC_{50} value, the higher toxicity of the sample.

Initial Microtox® Standard (Microbics Corporation) trials (sometimes referred to as Microtox® Acute Toxicity Test (Basic Test)) to determine an EC_{50} primary dilution sequence were unsuccessful. An alternative assay, the Microtox® Medical Device

Screening Protocol (MDSP), was used to assess non-relative toxicity of replicated pore water samples (Microbics Microtox® Manual). Luminescent bacteria, *Vibrio fischeri,* were exposed to non-diluted pore water samples in a replicate four-tube plus control series. The test endpoint, light emission inhibition, was determined at 15°C over 30 minutes, using a Microtox® M500 Toxicity Analyser and expressed as percentage light emission inhibition at neat (49.5%) pore water concentration. The higher the light emission inhibition value, the higher the toxicity of the sample.

The *Heliocidaris tuberculata* embryological development toxicity test, (Simon & Laginestra, 1997) was utilised to measure sublethal toxicity of pore water samples. This bioassay has been found to be extremely sensitive to a wide range of anthropogenic contaminants (Stauber *et al.*, 1996; Simon & Laginestra, 1997). *Heliocidaris tuberculata*, commonly found along the east coast of mainland Australia, exhibits a 10 month spawning season (Laegdsgaard *et al.,* 1991) and has been used effectively to determine the toxicity of complex effluents and ambient marine waters from February to early December (Simon & Laginestra, 1997). Several attempts to use this protocol with *Heliocidaris erythrogramma* (commonly found in Tasmanian waters) were unsuccessful due to a relatively narrow spawning period occuring over the summer months in Tasmanian waters (Williams & Anderson, 1975), and problems associated with premature spawning during collection, transportation and containment within tanks (Eriksen *et al.,* 2000).

Pore water samples for the *H. tuberculata* bioassay were stored at -20°C for transportation to AWT Ensight, Sydney, for analysis. Samples were thawed overnight (4°C) at AWT Ensight, prior to analysis. *H. tuberculata* assays were only conducted on samples collected during winter 1997. The test parameter, larval development abnormality, is regarded as environmentally relevant as a decrease in successful gamete development implies that survival of the population may be compromised. At the end of the 72h exposure, the fertilised eggs should have developed to the pluteus stage with both oral and post-oral arms developed. Abnormal development was defined as one or more of the following morphological characteristics:

- failure to develop to the pluteus stage

- one or more of the arms bent or unequal arm length within pair of arms
- internal skeleton not fully developed or evidence of breaks
- internal skeleton protruding more than one quarter of the total length of the arm
- larvae significantly (by more than 25%) smaller than the average in the controls
- any other obvious deformity.

The test data are reported as effective pore water concentration eliciting a 50 % reduction in successful gamete development over 72 hours (72 hour EC_{50} larval abnormality). The lower the EC_{50} value, the higher the toxicity of the sample.

Benthic macroinvertebrate analysis

Sediment cores were collected as described above and washed through a graded series of Endecott sieves to 1mm using 0.4 μm filtered seawater with Rose Bengal. Organisms retained by the sieves were sorted under a dissecting microscope, preserved in 70% ethanol and identified to species level or as close to this level as possible. Indices of benthic community structure and diversity measures were assessed from four pooled sediment cores per site (490 cm^3). Estimates of the number of individuals and taxa, species richness (Margalef's), evenness (Pielou's *J'*), dominance (Simpson) and diversity (Shannon-Wiener *H'*) were determined using the computer software package PRIMER v4 (Plymouth Routines in Multivariate Ecological Research) developed at the Plymouth Marine Laboratory (Clarke & Warwick, 1994).

Statistical analysis

Algal growth inhibition EC_{50} values were calculated using the Dynamic Energy Budget model (DEB) computer package DEBtox (Kooijman & Bedaux, 1996). Sea urchin larval abnormality EC_{50} values were calculated by the trimmed Spearman-Karber method, using the ToxCalc computer package (ΣTidepool Scientific Software). The field survey design assessed spatial variability within and between sampling stations. Statistical comparisons between physicochemical, toxicity and benthic indices data were determined by analysis of variance (ANOVA) using the computer package JMP (SAS Institute Inc.,1995). Heterogeneous physicochemical and benthic macroinvertebrate data were log transformed. Arcsine square root transformation was used for toxicity data where necessary. If data

were intractably heterogenous after transformation, the data were tested non-transformed and the results were interpreted with caution (Underwood, 1981). If significant differences were detected, the Tukey-Kramer comparison of means test was used. A divisive quantitative hierarchical cluster analysis on pooled, non-transformed data sets of SQT component variables was also performed to provide a description of among-site patterns for each set of variables, followed by two-way contingency table analysis (JMP, SAS Institute Inc.,1995) to describe among-sets relationships (Green *et al.*, 1993). Variables from the chemistry, toxicity and biota (sediment quality triad (SQT) data set) were further analysed using Principal Components analysis (PRIMER, Plymouth Marine Laboratories, 1994) and linear regression (JMP, SAS Institute Inc.,1995). A correlation-based PCA of log transformed chemical data, and geochemical data were performed and presented as a two-dimensional PCA ordination. Correlation between the biotic data, and chemical and geochemcial variables involved linear regression of species diversity (Shannon diversity, *H'*), and toxicity against the first PC axis score from the respective chemical and geochemical PCAs (Clarke & Warwick, 1994).

Sediment quality triad differential analysis

Differences between the collective components of the SQT in the current study are based on statistically significant differences ($p<0.05$) detected within each component. Chemical contamination is indicated by greater chemical concentrations relative to reference sites, and where at least TEL or ER-L sediment quality guideline values were exceeded. Toxicity is indicated by a statistically significant reduction in biological endpoints indicative of reduced physiological success relative to reference sites. In the absence of historic benthic data for the study sites, difference in benthic assemblages is indicated by statistically significant differences in benthic diversity indices relative to reference sites. Species richness (Margalef's), which provides an accepted comprehensible measure of diversity (Magurran, 1983), was coupled with evenness (Pielou's *J'*) to represent differences in benthic community, rather than attempting to identify changes in community structure which is recognised to be relatively insensitive to pollution caused community changes (Wilson & Jeffrey, 1994).

Results

Granulometry

Both the Tamar River and Port Sorell localities exhibited medium to fine sandy sediments with similar mud to sand ratios (Table 1). However, the composition of mud differed significantly ($p<0.05$), with both Tamar River localities exhibiting a higher silt and lower clay proportion in mud than Port Sorell (Table 1). Total organic carbon was also significantly higher in the Tamar River estuary localities ($p<0.05$) relative to Port Sorell (Table 1). Temporal and spatial differences in granulometry and %TOC between sites within localities were non-significant.

Chemical analysis

Trace metal concentrations in Tamar River estuary sediments far exceeded values detected in Port Sorell (Table 1). Trace metal concentrations at Deceitful Cove (Al, Cd, Mn, Ni, Pb, and Zn) were significantly higher than at other localities. Manganese and Zn levels in particular were found at concentrations which were several orders of magnitude higher than Port Sorell (Table 1). Both Deceitful Cove and East Arm exhibited significantly elevated Cu and Al concentrations relative to Port Sorell, whilst East Arm exhibited the highest Cr and Fe levels (Table 1). Deceitful Cove displayed the greatest trace metal loading overall (Table 1).

Contamination by organics was predominantly confined to Deceitful Cove. Concentrations of PAHs were below detection limits (0.5 mg kg^{-1} dry weight) in all but one other locality. Pyrene (0.6 mg kg^{-1}) and benzo(*b*)fluoranthene (1.1 mg kg^{-1}) were detected in one sample from North East Arm (winter 1996). Seasonal variation in PAH contamination at Deceitful Cove was evident. Mean winter PAH values exceeded summer values for ten of the eleven compounds detected (Table 2). Five compounds present in winter (benzo(*k*)fluoranthene, benzo(*a*)pyrene, benzo(*ghi*)perylene, indeno(*1,2,3-cd*)pyrene and phenanthrene) were below detection levels during summer. The majority of organic compounds detected were high molecular weight PAHs.

Table 1. Mean (± S.E.) values for selected physicochemical parameters of sediment data: n = 128. Chemical data log transformed. Different letters indicate statistically significant differences ($p<0.05$) for each parameter.

Parameter	Deceitful Cove	East Arm	Squeaking Point	North East Arm
Granulometry				
Wentworth classification	medium to fine sand	medium to fine sand	medium to fine sand	medium to fine sand
% sand	87.12 ± 0.51	87.52 ± 0.6	87.2 ± 0.42	88.03 ± 0.32
% silt	8.87 ± 0.45	8.29 ± 0.53	5.9 ± 0.35	3.41 ± 0.32
% clay	3.97 ± 0.31	4.19 ± 0.05	6.93 ± 0.19	8.56 ± 0.21
% mud (silt + clay)	12.84 ± 0.5 ^{a}	12.48 ± 0.6 ^{a}	12.83 ± 0.42 ^{a}	11.97 ± 0.32 ^{a}
%TOC	16.4 ± 0.1.19 ^{a}	13.55 ± 0.82 ^{b}	7.04 ± 0.18 ^{c}	5.15 ± 0.27 ^{c}
Trace metals (*mg kg^{-1} dry wt.*)				
Ag	0.18 ± 0.04 ^{a}	0.11 ± 0.004 ^{ab}	0.04 ± 0.008 ^{b}	0.02 ± 0.007 ^{b}
Al	12660.8 ± 2643 ^{a}	16196.2 ± 175.5 ^{a}	3217.7 ± 156.7 ^{b}	2017.3 ± 109.8 ^{b}
Cd	1.93 ± 0.49 ^{a}	0.65 ± 0.04 ^{b}	0.06 ± 0.01 ^{b}	0.04 ± 0.008 ^{b}
Cr	14.6 ± 1.14 ^{b}	42.75 ± 1.98 ^{a}	10.44 ± 0.32 ^{bc}	8.62 ± 0.93 ^{c}
Cu	26.83 ± 3.1 ^{a}	20.62 ± 0.72 ^{a}	3.8 ± 1.25 ^{b}	0.93 ± 0.07 ^{b}
Fe	11195.7 ± 840.2 ^{b}	44376.8 ± 1063.7 ^{a}	6485.2 ± 272.5 ^{c}	3766.6 ± 170.9 ^{c}
Mn	57215.9 ± 10808 ^{a}	359.7 ± 44 ^{b}	45.1 ± 2 ^{b}	25.0 ± 2 ^{b}
Ni	75.1 ± 20.86 ^{a}	15.75 ± 0.313 ^{b}	3.07 ± 0.236 ^{b}	1.89 ± 0.17 ^{b}
Pb	322.28 ± 68.54 ^{a}	19.02 ± 0.6 ^{b}	8.4 ± 1.75 ^{b}	2.95 ± 0.16 ^{b}
Zn	1064.18 ± 235.16 ^{a}	126.75 ± 4.08 ^{b}	10.39 ± 0.98 ^{b}	7.9 ± 0.67 ^{b}
Σ trace metals	80862.4 ± 9618.7 ^{a}	51356.9 ± 4235.9 ^{b}	9412.7 ± 707.4 ^{c}	5698.2 ± 486.6 ^{c}

Table 2. Seasonal variation in mean (± S.E.) polycyclic aromatic hydrocarbon (PAH) concentrations in shallow subtidal sediment from Deceitful Cove. n=96. Different letters indicate statistically significant differences ($p<0.05$) between summer and winter for each PAH compound detected.

Polycyclic aromatic hydrocarbons ($\mu g\ kg^{-1}$ *dry wt.*)	Season	
	Summer	Winter
Anthracene	75.0 ± 75.0^{a}	0^{b}
Benzo(*a*)anthracene	87.5 ± 87.5^{b}	916.67 ± 279.6^{a}
Benzo(*b*)fluoranthene	68.75 ± 68.75^{b}	1183.0 ± 263.11^{a}
Benzo(*k*)fluoranthene	0^{b}	375.0 ± 95.4^{a}
Benzo(*a*)pyrene	0^{b}	783.3 ± 180.34^{a}
Benzo(*ghi*)perylene	0^{b}	191.67 ± 88.65^{a}
Chrysene	31.25 ± 31.25^{b}	454.2 ± 159.03^{a}
Fluoranthene	581.25 ± 320.57^{b}	2041.0 ± 711.29^{a}
Indeno(*1,2,3-cd*)pyrene	0^{b}	179.17 ± 77.55^{a}
Phenanthrene	0^{b}	225.0 ± 100.3^{a}
Pyrene	418.75 ± 227.89^{b}	1445.83 ± 425.16^{a}

Twelve contaminants and contaminant mixtures in the Tamar River estuary locations exceeded SQG values, the majority of cases occurring in Deceitful Cove sediment samples (Table 3). Lead, Ni, and Zn exhibited the highest metals concentrations, surpassing the ER-M values of 218, 51.6 and 270 mg kg^{-1} dry wt. for Pb, Ni and Zn respecitively (Long *et al.* 1995). Benzo(*a*)pyrene, crysene, fluoranthene and $\sum$ HMW PAHs exhibited the highest organics levels in winter samples, exceeding the ER-M values of 1600, 2800 , 5100 and 9600 µin kg^{-1} dry wt. respectively (Long *et al.*, 1995). Zinc concentrations at East Arm exceeded the Zn TEL and ER-L values of 120 and 120 mg kg^{-1} dry wt. respectively (Long *et al.* 1995; MacDonald et al. 1996). The mean SQG quotient for Deceitful Cove ranged between 1.76 (metals only) to 1.88 (metals +PAHs). This value excludes SQG quotients for chemicals which did not in exceed their corresponding ER-M value and were not included in Table 3: Ag, Cr and Cu.

Table 3. **Key contaminants detected at sites within the Tamar River estuary exceeding the threshold effect level (TEL), probable effects level (PEL) (MacDonald *et al.*, 1996), effects range-low (ER-L) and effects range-median (ER-M) values (Long *et al.* 1995). Mean sample concentration rounded to the nearest decimal point. SQG quotient: mean sample concentration normalised to (divided by) the ER-M SQGs (Long *et al.* 1998). n = 128. *Polycyclic aromatic hydrocarbon carbon (PAH) values for winter only, n = 96. Sum of low molecular weight PAHs [a]: anthracene and phenanthrene . Sum of high molecular weight PAHs [b]: benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)flouranthene, benzo(*a*)pyrene, benzo(*ghi*)perylene, indeno(*1,2,3-cd*)pyrene, chrysene, fluoranthene and pyrene. DC = Deceitful Cove, EA = East Arm, SP = Squeaking Point, NA = North East Arm.**

Contaminant	TEL	PEL	ER-L	ER-M	Localities exceeding TEL or ER-L / PEL or ER-M		Mean sample conc. detected per location	SQG quotient
Trace metals ($mg\ kg^{-1}$)								
Cd	0.7	9.6	1.2	9.6	DC	—	2	0.21
Pb	30	218	46.7	218	DC	DC	322	1.48
Ni	15.9	51.6	20.9	51.6	DC	DC	75	1.45
Zn	124	410	120	270	DC	DC	1064	3.94
					EA	—	127	0.47
PAHs ($\mu g\ kg^{-1}$)*								
Phenanthrene	86.7	544	240	1500	DC	—	225	0.15
Benzo(*a*)anthracene	74.8	693	261	1600	DC	DC	917	0.57
Benzo(*a*)pyrene	88.8	763	430	1600	DC	DC	7837	4.89
Chrysene	108	846	384	2800	DC	DC	4547	1.62
Fluoranthene	113	1494	600	5100	DC	DC	20417	4.0
Pyrene	153	1398	665	2600	DC	DC	1446	0.56
Σ LMW PAHs [a]	—	—	552	3160	DC	—	1440	0.46
Σ HMW PAHs [b]	655	6676	1700	9600	DC	DC	12113	1.26

Bioassays

The sea urchin larval abnormality test exhibited the greatest sensitivity to pore water samples. *H.tuberculata* EC_{50} larval abnormality values indicated varying degrees of pore water toxicity in both estuaries. Urchin larvae exhibited the greatest sensitivity to Deceitful Cove pore water, followed by decreasing sensitivities to East Arm and Squeaking Point samples. *H.tuberculata* gametes were least sensitive to pore water from North East Arm (Table 4).

Growth inhibition of the marine diatom *N.closterium,* differed significantly between locations. Seventy-two hour EC_{50} values for Deceitful Cove and East Arm exhibited an inhibition in algal growth not present in Port Sorell samples. Squeaking Point and North East Arm displayed enhanced rather than reduced algal growth indicative of non-toxic conditions (Table 4). No spatial or temporal variation was evident between sites within locations.

Vibrio fischeri was the least sensitive assay species. Physiological impairment induced by pore water exposure did not exhibit an EC_{50} value. Microtox® MDSP % light emission inhibition levels differed significantly between Tamar River locations and those at Port Sorell (Table 4). Exposure to Deceitful Cove pore water elicited the strongest inhibition. Both Port Sorell localities displayed enhanced light emission levels indicative of non-toxic conditions. No spatial or temporal variation was evident between sites within locations.

Table 4. **Mean (± S.E.) values for toxicity bioassay data for each site within survey location: n=124, * indicates n=32. Different letters indicate statistically significant differences ($p<0.05$) in toxicity between locations. Sampling sites within each location are designated by the numerals 1-4.**

Locality / sampling sites	Bioassay		
	Microtox® MDSP % light emission reduction	*Nitzchia closterium* 72 hour EC_{50} growth inhibition	*Heliocidaris tuberculata* 72 hour EC_{50} larval abnormality *
Deceitful Cove			
1	31.53 ± 2.85^{a}	42.77 ± 7.94^{a}	16.1 ± 1.7^{a}
2	24.98 ± 2.46^{ab}	45.68 ± 5.42^{a}	14.5 ± 1.9^{a}
3	26.19 ± 2.47^{ab}	34.90 ± 7.61^{a}	14.7 ± 0.9^{a}
4	25.12 ± 2.41^{ab}	41.86 ± 7.73^{a}	14.7 ± 2.6^{a}
East Arm			
1	19.35 ± 2.08^{b}	35.11 ± 4.47^{a}	49.4 ± 1.0^{b}
2	14.47 ± 0.83^{b}	34.99 ± 6.44^{a}	51.3 ± 2.3^{b}
3	11.41 ± 0.63^{b}	38.62 ± 8.08^{a}	44.3 ± 1.5^{b}
4	16.03 ± 1.04^{b}	29.63 ± 5.54^{a}	50.5 ± 2.2^{b}
Squeaking Point			
1	0^{c}	$>100^{b}$	62.6 ± 2.8^{c}
2	0^{c}	$>100^{b}$	66.0 ± 1.8^{c}
3	0^{c}	$>100^{b}$	62.8 ± 3.5^{c}
4	0^{c}	$>100^{b}$	58.5 ± 1.0^{c}
North East Arm			
1	0^{c}	$>100^{b}$	$>100^{d}$
2	0^{c}	$>100^{b}$	$>100^{d}$
3	0^{c}	$>100^{b}$	$>100^{d}$
4	0^{c}	$>100^{b}$	$>100^{d}$

Benthic macroinvertebrate analysis

Macroinvertebrate assemblages in the Tamar River and Port Sorell estuaries were made up of predominantly euryhaline marine and estuarine benthic species (Mondon *et al.*, *Unpublished*). Measures of species abundance, richness, evenness and diversity were highest in Port Sorell sediment samples (Table 5). North East Arm exhibited the greatest number of individuals and taxa present, and the highest species richness and evenness values. Additionally, North East Arm displayed similar diversity and dominance values to those of Squeaking Point. Conversely, species dominance was highest in Deceitful Cove and East Arm in the Tamar River.

Table 5. Mean (± S.E.) values for benthic macroinvertebrate community descriptive parameters: n=128; pooled $\log_e$ transformed number of individuals and taxa, species richness, diversity, evenness and dominance / 450cm^3 sediment. Different letters indicate statistically significant differences ($p<0.05$).

Community parameters	Locality			
	Deceitful Cove	East Arm	Squeaking Point	North East Arm
No. individuals	98.75 ± 9.2 ^{a}	87.5 ± 4.2 ^{a}	135.75 ± 7.94 ^{b}	206.75 ± 10.94 ^{c}
No. taxa	20.5 ± 1.75 ^{a}	24.0 ± 1.58 ^{a}	29.5 ± 0.87 ^{b}	38.7 ± 0.63 ^{c}
Richness (Margalef)	4.25 ±0.32 ^{a}	5.14 ± 0.3 ^{a}	5.81± 0.13 ^{b}	7.09 ± 0.13 ^{c}
Diversity (*H'*)	2.29 ± 0.07 ^{a}	2.48 ± 0.08 ^{a}	2.96 ± 0.03 ^{b}	3.03 ± 0.09 ^{b}
Evenness (*J'*)	20.5 ± 1.75 ^{a}	24.0 ± 1.58 ^{a}	29.5 ± 0.87 ^{c}	38.7 ± 0.63 ^{b}
Dominance (Simpson)	0.18 ± 0.01 ^{a}	0.16 ± 0.02 ^{a}	0.07 ± 0.002 ^{b}	0.08 ± 0.01 ^{b}

Hierarchical cluster and contingency table analysis

The four-cluster solution forced on each set of pooled variables indicates a clear differentiation between Tamar River (Deceitful Cove and East Arm) and Port Sorell (Squeaking Point and North East Arm) localities based on SQT components (Table 6). Two-way contingency analysis of cluster sets demonstrate significant interaction between chemical loading and toxicity ($p = 0.0002$), chemical loading and species richness ($p<0.0001$), and species richness and toxicity ($p = 0.001$).

Table 6. Hierachical cluster analysis on pooled SQT components. A four-cluster solution was forced for each set of pooled variables: n=16. Cluster codes (1-4) are arbitrary and do not correspond between variable sets. SQT components: Chemistry = metal + PAH concentrations; Toxicity = Microtox® MDSP % light emission reduction; Biota = species richnes. Sampling sites within locations are designated by the numbers 1-4.

Locality	Cluster membership for SQT variable sets		
	Chemical	Toxicological	Biological
Deceitful Cove			
1	1	1	1
2	1	2	2
3	1	2	1
4	1	2	2
East Arm			
1	2	3	2
2	2	3	2
3	2	3	2
4	2	3	2
Squeaking Point			
1	3	4	3
2	3	4	3
3	3	4	3
4	3	4	3
North East Arm			
1	3	4	4
2	3	4	4
3	3	4	4
4	3	4	4

PCA and linear regression analysis

The 2-dimensional PCA ordination of log-transformed chemical concentration data suggests separation of the data into three distinct sets of samples: Deceitful Cove, East Arm, and Squeaking Point + North East Arm (Figure 2a). The first two PC axes account for 95% of the total variance (65% for PC1 and 30% for PC2), providing an accurate summary of the sample relationships. PC1 represents an axis of increasing contaminant load (Table7).

Table 7. Co-efficients in the linear combinations of log-transformed chemical variables making up principal components: n=128; * indicates n=96

	PCA1	PCA2	PCA3
PAHs*	0.305	-0.220	0.413
Al	0.261	0.34	0.225
Mn	0.32	0.504	-0.133
Fe	0.09	0.504	-0.133
Ni	0.341	-0.123	-0.29
Cu	0.326	0.163	0.03
Zn	0.341	-0.163	-0.54
Ag	0.347	0.531	-0.41
Cd	0.35	-0.068	-0.279
Pb	0.334	-0.187	0.004
Cr	0.091	0.498	-0.18
NH_3 N	0.165	0.429	0.483
Eigenvalues	7.75	3.61	0.28
% Variation explained	65	30	2.3

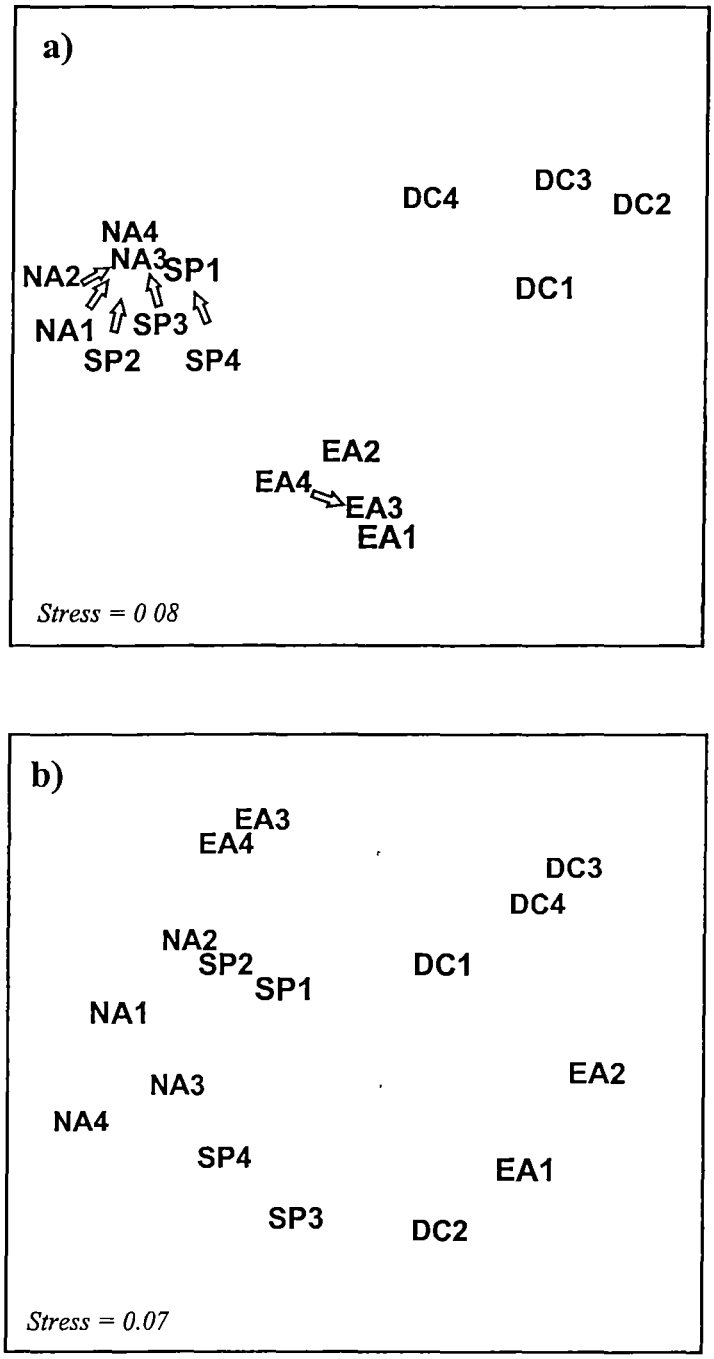


Figure 2. Two-dimensional PCA of survey sites based on environmental variables at locations in the Tamar River and Port Sorell estuaries: a) log-transformed trace metals, PAHs and NH_3 N; b) %TOC, %mud and salinity. DC = Deceitful Cove, EA = East Arm, SP = Squeaking Point, NA = North East Arm.

The 2-dimensional PCA ordination of %TOC, % mud and salinity suggests separation of data into three relatively poorly defined sets of data: Deceitful Cove, East Arm and Squeaking Point + North East Arm (Figure 2b). The first two axes account for 88% of the variation (58% for PC1 and 30% for PC2). Broadly, speaking, PC1 represents an axis of increasing geochemical concentrations (Table 8).

Table 8. Co-efficients in the linear combinations of geochemical variables making up principal components. n=128.

	PCA1	PCA2	PCA3
%mud	0.326	0.945	-0.03
%TOC	0.671	-2.09	0.711
salinity	-0.665	0.252	0.703
Eigenvalues	1.74	0.91	0.35
% Variation explained	58	30.4	11.6

Species richness, evenness and toxicity varied as a linear function of overall chemical concentration. Both species richness (Margalef) and evenness (J') decreased with increasing chemical loading (PC1 axis score) (Figure 3a & 3b), and toxicity increased with increasing levels of contamination (Figure 3c). Similarly, species richness, evenness and toxicity varied as a linear function of measured geochemical variables, with species richness and eveness decreasing, and toxicity increasing, with combined increases in %TOC and % mud, and decreased salinity (Figure 4a, b & c).

Sediment quality triad differential analysis

Both Deceitful Cove and East Arm on the Tamar River estuary exhibited elevated toxicity and sediment contaminant levels, plus lower benthic species diversity, compared to reference localities at Port Sorell (Table 9). The sea urchin growth inhibition assay detected toxicity in pore water samples from one of the reference locations (Squeaking Point) resulting in a positive classification for the toxicity component of the differential analysis.

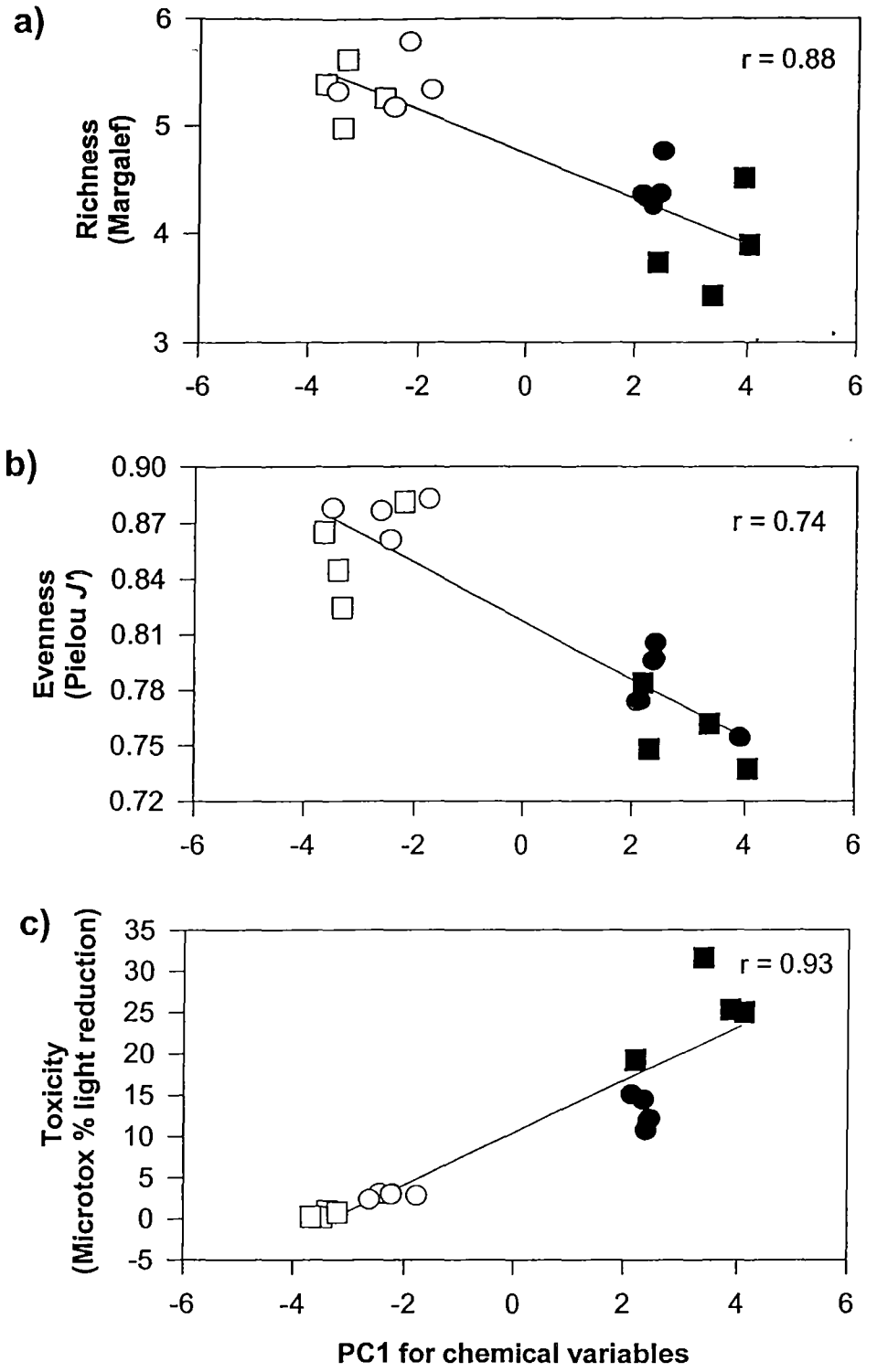


Figure 3. Linear regression of species richness, evenness and toxicity at sampling sites from the Tamar River and Port Sorell estuaries, against the first PC axis score from the trace metals, PAHs and NH_3 N PCA of Figure 2a: a) Species richness (Margalef), b) Evenness (Pielou *J'*), c) Microtox® toxicity. (■) = Deceitful Cove, (●) = East Arm, (O) = Squeaking Point, (□) = North East Arm.

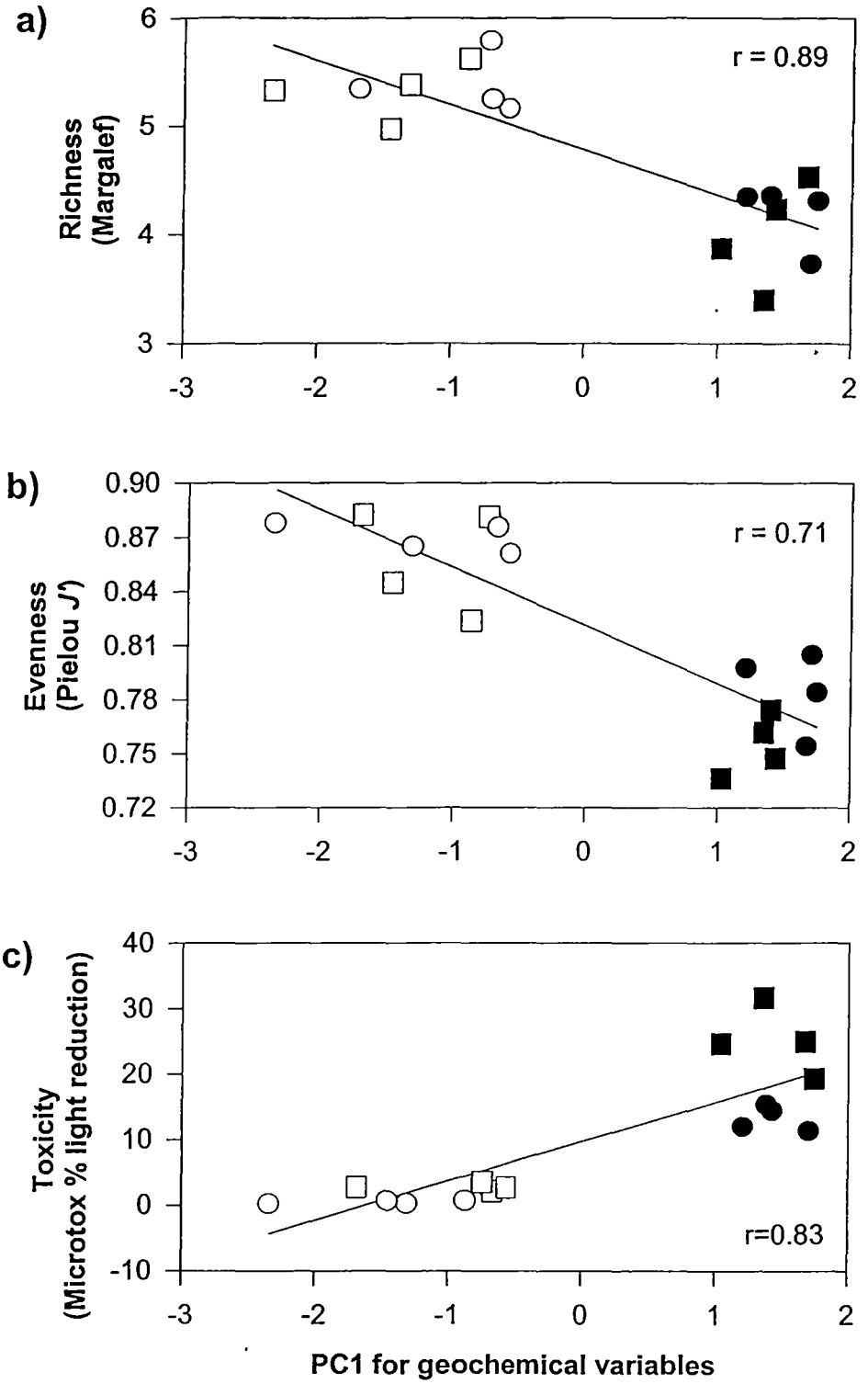


Figure 4. **Linear regression of species richness, evenness and toxicity at sampling sites from the Tamar River and Port Sorell estuaries, against the first PC axis score from the %TOC, %mud and salinity PCA of Figure 2b: a) Species richness (Margalef), b) Evenness (Pielou *J'*), c) Microtox® toxicity. (■) = Deceitful Cove, (●) = East Arm, (O) = Squeaking Point, (□) = North East Arm.**

Table 9. Summary of sediment quality triad data (adapted from Chapman, 1990)

Chemistry: + indicates the detection of a sediment bound contaminant concentration exceeding either the PEL or ER-M value, and the mean SQG quotient > 1; +/- indicates the detection of a sediment bound contaminant concentration exceeding either the TEL or ER-L value (Long *et al.* 1995; Long & Wilson, 1997).

Toxicity: + indicates a significant reduction in physiological activity in one of three moderately to sensitive bioassays (Microtox® % light emission reduction, algal EC_{50} growth inhibition, sea urchin EC_{50} larval abnormality.

Benthos: + indicates a significant difference in species richness and evenness relative to the Port Sorell reference locations.

Chemistry	Toxicity	Benthos	Locality	Possible conclusions
+	+	+	Deceitful Cove, Tamar River estuary	Evidence of pollution-induced degradation
+/-	+	+	East Arm, Tamar River estuary	Evidence of contaminant related stress
–	+	–	Squeaking Point, Port Sorell estuary	Unmeasured chemicals or conditions may exist with the potential to cause degradation
–	–	–	North East Arm, Port Sorell estuary	Strong evidence that there is no pollution-induced degradation

Discussion

The findings indicate considerable differences in sediment quality parameters between the localities in the Tamar River and Port Sorell estuaries. In all cases the differences show poorer sediment quality at the Tamar River estuary sites.

Chemistry

Trace metal concentrations were elevated in the Tamar River estuary, with total chemical loading in Deceitful Cove exceeding all other sites. Given the history of industrial effluent release into Deceitful Cove the levels are not unexpected. A similar pattern of sediment-bound metals was noted in 1990-91 (Gawne & Richardson, 1992). Except for Fe, trace metal concentrations in Deceitful Cove sediments were significantly higher than levels recorded elsewhere in the lower Tamar River estuary. Despite the potential "field errors" associated with spatial and temporal comparison of contaminant concentrations (Krumgalz *et al.*, 1989), the overall mean concentrations of Al, Fe, Pb, Mg and Zn in the current study are within the mean range detected in 0-10 cm shallow subtidal core samples collected by Gawne and Richardson in 1990-1991 (Gawne & Richardson, 1992) at the same subtidal sites. The current levels suggest that trace metal concentrations are as high as they were in 1990, implying sediments within the Cove are still a significant reservoir of metals (Gawne & Richardson, 1992). Further, the data imply a stability of trace metal material within the cove with respect to re-suspension and active transport from the site. Elevated Fe levels detected at East Arm in the current survey were also present in the 1990-91 survey, indicating a source of Fe, either natural or otherwise, existing in this region (Gawne & Richardson, 1992).

Comparison between sediment bound PAH concentrations detected in winter 1990 (Gawne & Richardson, 1992) and current (1996-1997) levels points to an approximate 50 fold increase in shallow subtidal sediment PAH levels over a 6 to 7 year period at the same site. However, it is not known whether the current PAH levels mirror the historical pattern of higher intertidal concentrations occurring in the upper reaches of Deceitful Cove. Intertidal concentrations detected in 1990 were significantly higher than subtidal levels,

implying considerable input of PAHs via the storm water outlet (Gawne & Richardson, 1992).

A number of possibilities may account for seasonal variation in PAH levels at Deceitful Cove. The non-detection of PAHs in summer samples may be due to the warmer conditions and longer daylight hours potentially facilitating photooxidation of PAHs in the microlayer of shallow subtidal sediments (McKinney *et al.*, 1999). Additionally, potential loss of PAHs via the extraction process may also be responsible for non-detection (Schults *et al.*, 1992). Considering the highly elevated PAH concentrations detected in winter relative to summer values, and the previous findings (Gawne & Richardson, 1992), increased storm water run-off entering Deceitful Cove from the Bell Bay industrial estate is most likely to be responsible in elevating sediment PAH concentrations above detection levels during winter, possibly stemming from petroleum hydrocarbons, industrial site run-off, and combustion. Products of incomplete combustion by domestic wood heaters in the Tamar River valley are trapped by frequent thermal inversion layers during the winter months, and they are also a possible contributing factor responsible for the high proportion of high molecular weight PAHs present. A conservative estimate of greater than 200 tonnes of wood smoke per month is produced in the upper Tamar Valley during winter (Air Pollution., 1996). Local levels of particulate air pollution in the middle and upper reaches of the Tamar River estuary during the winter months have been recorded as high to very high for compounds such as benzo(*a*)pyrene (Air Pollution, 1996). The absence of PAHs in East Arm sediment in winter points to a local source at Deceitful Cove, as opposed to atmospheric fallout from domestic sources. However, the geochemistry of Deceitful Cove may be instrumental in preferentially collecting and storing PAHs relative to East Arm. While there is a non-significanct difference in middle silt fractions (which are thought to be primarily responsible for PAH transportation (Bierl & Umlauf, 1987) between Deceitful Cove and East Arm, the higher levels %TOC and Mn oxides at Deceitful Cove may provide a greater absorbtion area for organic contaminants than at East Arm (Lee *et al.*, 1992). Further investigative work on seasonal PAH variation, including atmospheric fallout and industrial stockpile sources, and photooxidation within surface sediments is necessary.

Sediment Quality Guidelines

SQGs facilitate evaluation of the potential toxicological relevance of contaminant concentrations within marine and estuarine sediments. Of the twelve contaminants that were in excess of the SQG values at Deceitful Cove, seven exceeded ER-M (effects range median) values. The probability of highly toxic responses occurring when one or more ER-M values are exceeded is 48%, when running a moderately sensitive assay, or 86% when using a battery of sensitive tests (Long *et al.*, 1998). Additional increases in the number of chemicals exceeding ER-M values further increases the likelihood of highly toxic responses. The probability of toxicity also increases with elevation in mean SQG quotients. Mean SQG quotients of 1.76 to 1.88 for Deceitful Cove are relatively high (Long & Wilson, 1997). Conservatively, the probability of a highly toxic response occuring for moderately sensitive species is 71% (Long *et al.*, 1998). An 86% or greater probability of exhibiting a highly toxic response is expected with sensitive species. Sediments in East Arm exceeded one ER-L value, indicating a 16% - 32% likelihood of highly toxic responses occurring based on SQG values and mean SQG quotients respectively, or 60% probability when applied to sensitive species (Long *et al.*, 1998). Predictions of sediment quality, based on SQGs and SQG quotients, point to the strong possibility of detrimental effects on sensitive species exposed to Deceitful Cove sediment. Further, the SQG values imply that the degree of pollution at Deceitful Cove potentially far exceeds that of East Arm. A preliminary survey of polychlorinated biphenyl (PCBs) contamination of shallow subtidal sediments in the Tamar River and Port Sorell estuaries (1997), found PCB concentrations at Deceitful Cove in excess of the total PCB ER-M value of 180 ppb (dry wt.) (Mondon *et al.*, 1999),which increases the likelihood of a highly toxic response occurring at Deceitful Cove.

Bioassays

Statistically significant toxicity was observed in all bioassays for Deceitful Cove and East Arm pore water samples, and for Squeaking Point in the case of the sea urchin assay. Seasonal differences in toxicity were not detected. Unfortunately there are no historical sediment toxicity data available for comparison. Each assay closely tests pathways of uptake predominant in nature. Different taxa and bioassay endpoints exhibited varying susceptibility to contaminants present in the two estuaries, indicating differences in

contaminant specificity between test species. The sea urchin assay demonstrated the greatest sensitivity to contaminants which is not unexpected. Embryological development assays are regarded as highly sensitive relative to Microtox® pore water test system sensitivity (Carr, 1998).

Whilst the Microtox® MPSP bioassay detected measurable levels of toxicity at Deceitful Cove and East Arm, failure to elicit an EC_{50} value using the Microtox® Standard assay indicates a considerable lack of sensitivity to mixtures of predominantly trace metal contaminants. Various combinations of metal ions are known to exhibit a variety of synergistic, antagonistic and additive responses with the Microtox test (Qureshi *et al.*, 1984). Stimulation effects on light illumination observed during the first five minutes of Microtox® MPSP exposure to some pore water samples in the current study correlates with observations recorded from Microtox® Standard tests conducted on leachate extracted from steelworks effluent (Skacel *et al.*, 1993). It was noted in the steelworks study that short-term bacterial adaptation during the early phase of the test resulted in comparatively higher endpoint values, a response associated with predominantly metal-based contaminants rather than organic compounds (Skacel *et al.*, 1993). A time-dependent change in decreased toxicity was also noted in steelworks effluent leachate tested 42 hours after collection. As recommended guidelines on pore water storage (Carr & Chapman, 1995) were followed in the current study, it is unlikely storage time was a factor in reduced toxicity detected by Microtox®. It is also possible that toxicity may have been masked by metal sorption to organic carbon within samples (Tessier *et al.*, 1996). Both Deceitful Cove and East Arm exhibited higher organic carbon levels than Port Sorell sites.

Uptake of pore water-associated toxicants is thought to be a primary source of metal toxicity (Chapman *et al.*, 1998). Pore water assays estimate the potential toxicity of exposed or re-suspended sediments rather than simulating *in-situ* conditions. Removal of porewaters from sediments eliminates natural factors such as grain size, and suppresses the rate of change in redox conditions and metabolite concentrations within the samples (Chapman, 1989). Exposure of anoxic pore water to oxygen results in rapid precipitation of Fe oxides, whereby trace elements co-precipitating with the Fe oxides are removed from

solution. (Troup *et al.*, 1974). Consequently, oxygenating porewaters during extraction and assays may alter toxicity of samples (Chapman *et al.*, 1998), and therefore results may under-estimate metal toxicity due to this factor. However, whilst precipitation of toxicants is of importance as a potential source of false-negative responses in bioassays, oxidation of anoxic porewaters when exposed to surface waters in the field may not be deleterious. Reduced porewaters with elevated concentrations of reduced Fe and Mn in solution may not impact greatly on the adjacent ecosystem if exposure to oxygen through a sediment disturbance, such bioturbation or storm activity, immobilises the potential toxicity of that pore water via the co-precipitation of metals.

The presence of small concentrations of Mn, and to a lesser extent Fe, in marine waters is known to alleviate copper toxicity to the marine diatom *Nitzchia closterium* (Stauber & Florence, 1985). Considering the high levels of Mn at Deceitful Cove and comparatively low levels of Cu relative to other locations, the inhibited growth rate at Deceitful Cove points to toxicity from contaminant sources other than Cu at this site. It is feasible that Mn and Fe as metal binding agents in pore water (Smith & Jenne, 1991) could also alleviate toxicity from other metals present in test samples. The *N. closterium* test results demonstrate low levels of potentially toxic trace metals in pore water stimulated algal growth. Cadmium, for example, exhibits nutrient status for several species of phytoplankton at inorganic Zn concentrations typical of surface seawater (Lee, 1995). The growth inhibition of algae exposed to Deceitful Cove and East Arm porewaters, implies the higher metal concentrations in these samples exceed the diatom's threshold for at least one or more of the elements present.

Pore water samples from the Tamar River were clearly toxic in all bioassays, indicating that contaminants present in porewaters were in excess of biologically tolerated levels. As such, sites within this estuary could be considered to be polluted. Additionally, pore water assays only indicate relative toxicity to soluble contaminants. Some PAHs exert a photoinduced enhanced toxicity after accumulation of phototoxic PAHs (eg. flouranthene and pyrene) by benthic invertebrates (Swartz *et al.*, 1997). Conceivably, photoactivation

and enhanced toxicity by UV exposure may occur within shallow subtidal sediments at Deceitful Cove. Photoactivated toxicity is not detected by standard pore water bioassays.

Macroinvertebrate abundance and diversity

The premise for determining benthic assemblage change as a result of pollution is based on the tenet that parallel communities would exist where similar selective forces and responses occur (relating to predation or larval settlement, for example), and that similar groups of more or less closely related species would be found. The Port Sorell and Tamar River estuaries could be expected to display a similarity in infauna species abundance and composition in the lower, marine reaches of each estuary. Although the Tamar River represents a much larger river system in terms of catchment and drainage areas (Edgar *et al.*, 1999a), both estuaries are permanenetly open to the Bass Stait, and within 22 km of each other. Tidal ranges in the lower reaches of both estuaries exceeds 1 m in height, with a salinity range of shallow subtidal surficial pore water between 28 ppt - 36 ppt at low tide. Intertidal macroinvertebrate species composition is not influenced by the predominant rock type in the catchment area of either estuary (Edgar *et al.*, 1999b).

A change or difference in species diversity in parallel communities may provide a biological indicator of disturbance or contamination. The effect of trace metals in reducing benthic infauna abundance, richness and diversity has been demonstrated in a number of studies (Ahn *et al.*, 1995). Copper, Pb and Zn contaminated estuarine sediments in the Sydney region exhibit less diverse macrobenthic assemblages compared to unpolluted bays (Stark, 1998). Zinc contamination has the potential to profoundly affect the abundance and diversity of macro and meiofauna recruitment of sediment (Watzin & Roscigno, 1997). Geographically closer to the study site, a macroinvertebrate infauna survey in Macquarie Harbour, western Tasmania, found an inverse correlation between species diversity and Cu concentration (Talman *et al.*, 1996). Additionally, Cu concentration and sediment organic matter were the primary factors determining species richness, total abundance and distribution of benthic macroinvertebrates within Macquarie Harbour (Talman *et al.*, 1996).

In the absence of previous shallow subtidal benthic studies, it is difficult to establish whether the macroinvertebrate assemblages in the Tamar River estuary, and Deceitful Cove in particular, have changed over time, and whether that change is the result of long term sediment contamination. The significant difference in species diversity indices identified in the current survey does not inherently imply deleterious change. A recent study of Port Phillip Bay, Victoria, found significant changes in benthic community assemblages to have occurred over 20 years (Currie & Parry, 1999), possibly due to changes in pollutions levels. Conceivably the benthic infanuna of Deceitful Cove may have experienced a similar change in assemblage over the equivalent duration of time, given the corresponding increase in industrial activity, regional rural and urban development, shipping, recreational boating and fishing activity in the Port Dalrymple, Lower Tamar River estuary region.

Intertidal surveys conducted within similar areas of Tasmania identified allied species grouped by salinity regime (Smith, 1995). The pattern of declining species number with decreasing salinity (Edgar *et al.*, 1999b) does not explain the abundance pattern of shallow subtidal macroinvertebrates in the current study. Salinity levels at Deceitful Cove (31.68 ± 0.33) and North East Arm (32.67 ± 0.26) were similar, yet North East Arm supported almost twice the number of taxa. The macroinvertebrate data indicate lower species abundance and diversity within benthic assemblages in the Tamar River estuary compared to Port Sorell (Table 5) and other coastal embayments in Tasmania and south-eastern Australia (Poore & Rainer, 1979; Poore, 1982; Talman *et al.*, 1996; Edgar *et al.*, 1999b). Although the number of taxa present in the Tamar is not indicative of depauperate benthic assemblages, it has been suggested that a euryhaline region the size of the lower Tamar estuary could support anywhere from 40 to >100 species (Wilson & Jeffrey, 1994).

Although the benthic assemblage associated with present levels of contamination at Deceitful Cove is not indicative of a dramatic change having taken place, subtle changes in community structure related to pollution may be occurring, or may have occurred, and warrant further investigation. Where metals accumulate and persist in sediments, the potential for impact on benthic communities is high, particularly when "protracted press" disturbances, which are long term effects of contaminants, limit the recovery ability of

benthic assemblages (Glasby & Underwood, 1996). Diversity may have changed over the time course of contamination of the sediment. As bulk discharges into the bay have decreased to relatively infrequent disturbances compared to past input, it is possible that Deceitful Cove is in an intermediate phase of disturbance. Considerable changes in assemblages are often observed during the intermediate phase of disturbance, including a peak of species diversity (Connell, 1978). Decreased chemical input, in conjunction with fluctuations in natural environmental factors (salinity, temperature, ammonia), or subtle changes in granulometry, recruitment cycles, competition and predation, may be instrumental factors in the direction of change if it is, or has, occurred (Carriker *et al.* 1982). Further, it is possible that Deceitful Cove, following long-term contamination, contains phenotypically tolerant populations adapted to high metal concentrations (Depledge *et al.*, 1995), so that the current *in situ* effect of metal bioavailability on benthic infauna may not be detected using standard biodiversity monitoring methods.

Identifying the specific causes responsible for differences or alterations in benthic community structure is, however, not as important as the weight of evidence the data provide to establish the relationships among the triad components. The benthic data from this study provides evidence that the contamination and toxicity detected in Deceitful Cove sediment is of ecological relevance.

Contingency and Principle Components analysis

The contingency table analysis of the hierarchical cluster data highlights the strong possibility that chemical contaminant loading influences the benthic fauna assemblages due to toxicity associated with the chemicals present. This is in agreement with linear regression analysis results where an increase in toxicity and decrease in species diversity with increasing levels of contamination was indicated. Elevated NH_3N and TOC concentrations were also linked to decreased diversity and increased toxicity. The inverse relationship between %TOC and species diversity is particularly interesting in that it is contradictory to what might be found in the literature. Sediment organic matter is considered a primary food source, and inducement to settle, for benthic invertebrates, (Ahn *et al.*, 1995; Gray, 1966; Gray, 1967). Consequently increased species abundance and

richness would be expected where TOC is highest (Talman *et al.*, 1996). Toxicity and bioavailability of sediment-associated contaminants are generally inversely proportional to sediment TOC content (Carlberg *et al.*, 1986; Servos *et al.*, 1989; Lake *et al.*, 1990). However, uptake of contaminants may depend on the quality of organic material rather than quantity of TOC present (Gunnarsson *et al.*, 1999). Additionally, elevated TOC levels may also be indicative of high H_2S concentrations. The statistical associations in the current study indicate a direct relationship between elevated TOC and elevated toxicity. Total organic carbon levels may be collinear with inorganic contaminant concentrations, or naturally occurring hydrogen sulphide and / or pore water ammonia concentrations. Both Tamar River localities exhibit organically enriched sediments with proportionally higher silt contents which are conducive to ammonia-enriched sediments (Frazier *et al.*, 1996). Un-ionized ammonia levels detected in pore water samples exhibited a highly variable range at each locality: 0.06 - 0.42 mg/L (Deceitful Cove) and 0.09-0.58 mg/L (East Arm), Tamar River estuary; 0.10 - 0.22 mg/L (Squeaking Point) and 0.11-0.21 mg/L (North East Arm), Port Sorell. Although the association between toxicity and ammonia levels was inconclusive due to marked fluctuation between samples, peaks in ammonia concentration may elicit a localised toxic response in the field, whereby exposure is governed by variability of temporal and vertical distribution (Barbanti *et al.*, 1995) resulting from non-uniform *in situ* bioturbation and re-suspension processes (Krumgalz *et al.*, 1989). Lowest effects concentration (LOEC) values for ammonia for *H. tuberculata* is currently unknown, however, ammonia levels in the sea urchin assay porewaters exhibited a non-significant trend towards lower ammonia levels at Squeaking Point (0.13mg $\pm$ 0.04 mg/L) relative to North East Arm (0.18 $\pm$ 0.13 mg/L). This indicates that a factor other than ammonia may be responsible for the observed toxicity in Squeaking Point samples.

Although the relationships between overall chemical concentration and toxicity, and species diversity were strong, this does not necessarily imply causality. It is possible that biological effects could be the result of unmeasured anthropogenic variables with which the contaminant levels happen to correlate. Additionally, collinearity between environmental factors could be masking true associations between measured variables. However, PCA results do suggest that deleterious biological responses result from combined toxicity of

several contaminants whose individual components are present at sublethal concentrations, rather than a single chemical present at acutely toxic concentrations. In general terms, the data imply that anthropogenic contaminant concentrations caused toxicity in one or more tests and toxicity was accompanied by adverse measures of community structure.

Conclusions

In the worst case scenario, differential analysis of Deceitful Cove and East Arm indicated strong evidence of contaminant-induced degradation (Table 7). Based on this finding, remediation of contaminated sites in the lower Tamar River may be needed (Chapman, 1990). As we cannot conclude that there has been a deleterious change in macroinvertebrate assemblages, the alternative and far more conservative conclusion of strong evidence for contaminant related stress is also presented. The degree of degradation or stress between Deceitful Cove and East Arm cannot be differentiated within the SQT. Several metal and PAH concentrations exceeded the SQG values which suggests a higher level of degradation or stress occurring at Deceitful Cove. The possibility that unmeasured chemicals or conditions exist at one of the reference sites (East Arm) is also highlighted by the analysis, but this conclusion is based on a limited data set (sea urchin larval development), and is not definitive.

The SQT approach, combined with SQGs, has provided a means by which to determine the potential impact of predominantly trace metals toxicity on the ecology of a Tasmanian estuarine region of previously unknown ecotoxicological status. The study represents an initial screening of sediment quality and has identified several areas of concern where further work is warranted. Associations between chemical composition of shallow subtidal sediments and adverse biological effects detected imply that Deceitful Cove has been impacted by anthropogenic pollutants. Baseline data generated in this study will facilitate detection of changes in sediment chemistry, toxicity and benthic macroinvertebrate community alteration in future field assessments.

Acknowledgements

This study was funded by the Australian Research Council, TEMCO and Comalco Aluminium (Bell Bay) Limited. The authors thank Dr Graham Edgar, School of Zoology, University of Tasmania, and Dr Brian Smith, Queen Victoria Museum, Launceston, Tasmania, for assistance with macroinvertebrate identification; Ms Ruth Eriksen, CSIRO Marine Laboratories, Hobart, and Dr Jill Woodworth, formerly AWT Ensight, Sydney, for assistance with sea urchin analysis; Mr Jon Bryan, SeaNature Photography, and Ms Pollyanna Hilder, Mr Mark Hilder, Ms Susan Duda, Ms Marianne Helders and Mr Rob Gurney, School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, for their technical assistence in the field. Trace metal analyses of sediments were performed by Dr Ashley Townsend, Central Science Laboratory, University of Tasmania. Organics analysis of sediments were performed by Australian Environmental Laboratories, Hawthorn, Victoria.

References

Ahn, I., Kang, Y. & Choi, J. (1995). The influence of industrial effluents on intertidal benthic communities in Panweol, Kyeonggi Bay (Yellow Sea) on the west coast of Korea. *Marine Pollution Bulletin., 30*, 200-206.

Air Pollution, Environmental Health & Respiratory Diseases Working Party (1996). *Air Pollution, Environmental Health & Resiratory Diseases, Launceston and Upper Tamar Valley. Tasmania 1991-1994.*

Barbanti, A., Bergamini, M. C., Frascari, E., Miserocchi, S., Ratta, M. & Rosso, G. (1995). Diagenetic processes and nutrient fluxes @ the sediment-water interface, North Adriatic Sea, Italy. *Marine and Freshwater Research, 46*(1), 55-67.

Besser, J. M., Giesy, J. P., Kubitz, J. A., Verbrugge, D. A., Coon, T. G. & Braselton, W. E. (1996). Assessment of sediment quality in dredged and undredged areas of the Trenton channel of the Detroit River, Michigan USA, using the Sediment Quality Triad. *Journal of Great Lakes Research, 22*(3), 683-696.

Bierl, R. & Umlauf, G. (1987). Distribution of organic micropollutants in diferent size fractions of sediment and suspended solid particles of the River Rotmain. *Zeitschrift fuer Wasser und Abwasserforschug, 20*, 203-209.

Canfield, T. J., Brunsen, E. L., Dwyer, F. J., Ingersoll, C. G. & Kemble, N. E. (1998). Assessing sediments from upper Mississippi River navigational pools using a benthic invertebrate community evaluation and the Sediment Quality Triad approach. *Archives of Environmental Contamination and Toxicology, 35*(2), 202-212.

Canfield, T. J., Dwyer, F. J., Fairchild, J. F., Haverland, P. S., Ingersoll, C. G., Kemble, N. E., Mount, D. R., Lapoint, T. W., Burton, G. A. & Swift, M. C. (1996). Assessing contamination in Great Lakes sediments using benthic communities and the Sediment Quality Triad approach. *Journal of Great Lakes Research, 22*(3), 565-583.

Canfield, T. J., Kemble, N. E., Brumbaugh, W. G., Dwyer, F. J., Ingersoll, C. G. & Fairchild, J. F. (1994). Use of benthic invertebrate community structure and the sediment quality triad to

evaluate metal-contaminated sediment in the Upper Clark Fork River, Montana. *Environmental Toxicology and Chemistry, 13*(12), 1999-2012.

Carlberg, G. E., Martinsen, K., Kringstad, A., Gjessing, E., Grande, M., Kallqvist, T. & Skare, J. U. (1986). Influence of aquatic humus on the bioavailability of chlorinated micropollutants in Atlantic salmon. *Archives of Environmental Contamination & Toxicology, 15*, 543-548.

Carr, R. S. (1998). Marine and Estuarine Pore water Toxicity Testing. In P. G. Wells, K. Lee, & C. Blaise (Eds.), *Microscale Testing in Aquatic Toxicology: Advances, techniques, and Practice* . London: CRC Press.

Carr, R. S. & Chapman, D. C. (1995). Comparison of methods for conducting marine and estuarine sediment pore water toxicity tests - extraction, storage and handling techniques. *Archives of Environmental Contamination and Toxicology, 28*, 69-77.

Carr, R. S., Chapman, D. C., Howard, C. L & Biedenbach, J. M. (1996). Sediment Quality Triad assessment survey of the Galveston Bay, Texas system. *Ecotoxicology, 5*(6), 341-364.

Carr, R. S., Williams, J. W. & Fragata, C. T. B. (1989). Development and evaluation of a novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus*. *Environmental Toxicology & Chemistry, 8*, 533 - 543.

Chapman, P. M. (1989). Review: current approaches to developing sediment quality criteria. *Environmental Toxicology & Chemistry, 8*(589-599).

Chapman, P. M. (1990). The Sediment Quality Triad approach to determining pollution-induced degradation. *Science of the Total Environment, 97/98*, 815-825.

Chapman, P. M., Cano, M., Fritz, A. T., Gaudet, C., Menzie, C. A., Sprenger, M. & Stubblefield, W. A. (1997). Workshop summary report on contaminated site cleanup decisions. In C. G. Ingersol, T. Dillon, & G. R. Biddinger (Eds.), *Ecological Risk Assessment of Contaminated Sediments* (pp. 83-114). Pensacola: SETAC Press.

Chapman, P. M., Dexter, R. N. & Long, E. R. (1987). Synoptic measures of sediment contamination, toxicity and infaunal community composition (the Sediment Quality Triad) in San Francisco Bay. *Marine Ecology Progress Series, 37*, 75-96.

Chapman, P. M., Paine, M. D., Arthur, A. D. & Taylor, L. A. (1996). A Triad study of sediment quality associated with a major, relatively untreated marine sewage discharge. *Marine Pollution Bulletin, 32*(1), 47-64.

Chapman, P. M., Wang, F., Janssen, C., G., P. & Allen, H. E. (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability , risk assessment, and remediation. *Canadian Journal of Fisheries & Aquatic Science, 55*, 2221-2243.

Clarke, K. R. & Warwick, R. M. (1994). *Change in marine communities: an approach to statistical analysis and interpretation*. Plymouth: Plymouth Marine Laboratory.

Connell, J. H. (1978). Diversity in tropical rainforests and coral reefs. *Science, 199*, 1302-1310.

Currie, D. R. & Parry, G. D. (1999). Changes in benthic communities over 20 years in Port Phillip Bay, Victoria, Australia. *Marine Pollution Bulletin., 38*(1), 36-43.

Delvalls, T. A. & Chapman, P. M. (1998).Site specific sediment quality values for the gulf of Cadiz (Spain) and San Fransisco Bay (USA), using the Sediment Quality Triad and multivariate analysis. *Ciencias Marinas. 24, 3*(313-336).

Depledge, M. H., Aagaard, A. & Gyorkos, P. (1995). Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. *Marine Pollution Bulletin, 31*(1-3 Special issue SI), 19-27.

Edgar, G. J., Barrett, N. S., Graddon, D. J. & Last, P. R. (1999a). The conservation significance of estuaries: a classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. *Biological Conservation. In press.*

Edgar, G. J., Barrett, N. S. & Last, P. R. (1999b). Biogeography of Tasmanian estuaries. *In Press.*

Eriksen, R., Nowak, B. & van Dam, R. (2000). Copper speciation and toxicity in estuarine environments. *Unpublished.*

Frazier, B. E., Naimo, T. J. & Sandheinrich, M. B. (1996). Temporal and vertical distribution of tatoal ammonia nitrogen in sediment pore water form the upper Mississippi River. *Environmental Toxicology and Chemistry, 15*(2), 92-99.

Gawne, K. M. & Richardson, B. J. (1992). *An investigation into the distribution and accumulation of selected chemical species in the waters, sediments and biota of the lower Tamar estuary, Tasmania* : Deakin University Centre for Biological and Chemical Research.

Glasby, T. M. & Underwood, A. J. (1996). Sampling to differentiate between pulse and press perturbations. *Environmental Monitoring & Assessment, 42*, 241-252.

Gray, J. (1967). Substrate selection by the archiannelid *Protodrilus hypoleucus* Armenante. *Journal of Experimental Marine Biology & Ecology., 1*, 47-54.

Green, R. H., Boyd, J. M. & MacDonald, J. S. (1993). Relating sets of variables in environmental studies: the Sediment Quality Triad as a paradigm. *Environmetrics, 4*(4), 439-457.

Gunnarsson, J. S., Granberg, M. E., Nilsson, H. C., Rosenberg, R. & Hellman, B. (1999). Influence of sediment-organic matter quality on growth and polychlorobiphenyl bioavailability in Echinodermata (*Amphiura filiformis*). *Environmental Toxicology and Chemistry., 18*(7), 1534-1543.

Ingersoll, C. G., Ankley, G. T., Baudo, R., Burton, G. A., Lick, W., Luoma, S. N., MacDonald, D. D., Reynoldson, T. B., Solomon, K. R., Swartz, R. C. & Warren-Hicks, W. J. (1997). Workgroup summary report in uncertainty evaluation of measurement endpoints used in sediment ecological risk assessments. In C. G. Ingersoll, T. Dillon, & G. R. Biddinger (Eds.), *Ecological risk assessment of contaminated sediments.* (pp. 297-352). Pensacola: SETAC Press.

Kooijman, S. A. L. M. & Bedaux, J. J. M. (1996). *The analysis of aquatic toxicity data.* Amsterdam: VU University Press.

Krumgalz, B. S., Fainshtein, G., Sahler, M. & Gorfunkel, L. (1989). "Field error" related to marine sediment contamination studies. *Marine Pollution Bulletin, 20*(2), 64-69.

Laegdsgaard, P., Byrne, M. & Anderson, D. T. (1991). Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *Heliocidaris tuberculata* (Echinoidea) in New South Wales. *Marine Biology, 110*, 359-374.

Lake, J. L., Rubinstein, N. I., Lee, H. I., Lake, C. A., Helteshe, J. & Pavignano, S. (1990). Equalibrium partitioningand bioaccumulation of sediment-associated contaminants by infaunal organisms. *Environmental Toxicology and Chemistry, 9*, 1095-1106.

Lee, J., Chen, B., Allen, H. E., Huang, C. P., Sparks, D. L. & Sanders, P. (1992). Trace metal soil quality criteria to protect ground water. *Water Science and Technology, 26*(2327-2329).

Lee, J. G. (1995). *Cadmium: a toxin and a nutrient for marine phytoplankton.* Unpublished PhD., Massachusetts Institute of Technology, Woods Hole Oceanographic Institute.

Long, E. R. & Chapman, P. M. (1985). A Sediment Quality Triad: Measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Marine Pollution Bulletin, 16*(10), 405-415.

Long, E. R., Field, L. J. & MacDonald, D. D. (1998). Predicting toxicity in marine sediments with numerical sediment quality guidelines. *Environmental Toxicology and Chemistry, 17*(4), 714-727.

Long, E. R., MacDonald, D. D., Smith, S. L. & Calder, F. D. (1995). Incidence of adverse effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management., 19*, 81-97.

Long, E.R & Wilson, C.J. (1997). On the identification of toxic hot spots using measures of the sediment quality triad. *Marine Pollution Bulletin, 34*(6), 373-374.

Luoma, S. N. (1990). Processes affecting metal concentrations in estuarine and coastal marine sediments. In R. W. Furness & P. S. Rainbow (Eds.), *Heavy metals in the marine environment* (pp. 51-56). Boca Raton: CRC Press.

MacDonald, D. D., Carr,R.S., Calder,F.D., Long,E.R. & Ingersoll,C.G. (1996). Development and evaluation of sediment quality guidelines for Florida coastal waters. *Ecotoxicology, 5*,253-278.

Magurran, A.E. (1987) . *Ecological diversity and its measurement.* University Press, Cambridge.179pp.

Matthiessen, P., Blackburn, M., Kirby, M., Law, R., Sheahan, D. & Thain, J. (1995). Toxicity of estuarine waters and sediments resulting from industrial chemicals. *Marine Environmental Management Review of 1994 and Future Trends, 2*(4), 23-26.

McKinney, R. A., Pruell, R. J. & Burgess, R. M. (1999). Ratio of the concentration of anthraquinone to anthracene in coastal marine sediments. *Chemosphere, 38*(10), 2415-2430.

Mondon, J. A., Nowak, B. F. & Sodergren, A. (1999). Persistent organic pollutants in oysters *Crassostrea gigas* and sand flathead *Platycephalus bassensis* from Tasmanian estuarine and coastal waters. *Marine Pollution Bulletin. Accepted for publication.*

Paine, M. D., Chapman, P. M., Allard, P. J., Murdoch, M. H. & Minifie, D. (1996). Limited bioavailability of sediment PAH near an aluminium smelter - contamination does not equal effects. *Environmental Toxicology and Chemistry, 15*(11), 2003-2018.

Pascoe, G. A., Blanchet, R. J., Linder, G., Palawski, D., Brumbaugh, W. G., Canfield, T. J., Kemble, N. E., Ingersoll, C. G., Farag, A. & Dalsoglio, J. A. (1994). Characterization of ecological risks at the Milltown Reservoir Clark Fork River Sediments Superfund Site. *Environmental Toxicology & Chemistry, 13*(12), 2043-2058.

Phillips, D. J. H. (1993). Bioaccumulation. In P. Calow (Ed.), *Handbook of Ecotoxicology* (Vol. 2, pp. 378-396). London: Blackwell Scientific Press.

Poore, G. C. B. & Rainer, S. F. (1979). A three year study of benthos of muddy environments in Port Phillip Bay Victoria. *Estuarine and Coastal Marine Science, 9*(477-497).

Poore, G. C. B. (1982). Benthic communities in Gippsland Lakes, Victoria. *Australian Journal of Marine and Freshwater Research, 33*(901-905).

Qureshi, A. A., Coleman, R. N. & Paran, J. H. (1984). Evaluation and refinement of the Microtox test for use in toxicity screening. In D. Liu & B. J. Dutka (Eds.), *Tocicity procedures using bacterial systems.* (pp. 1-22). New York: Marcel Dekker.

Schults, D. W., Ferraro, S. P., Smith, S. P., Roberts, L. M & Poindexter, C. K. (1992). A comparison of methods for collecting interstitial water for trace organic compounds and metal analyses. *Water Research, 26*, 989.

Seelye, J. G., Hesselberg, R. J. & Mac, M. J. (1982). Accumulation by fish of contamination released from dredged sediments. *Environmental Science & Technology, 16*, 459-463.

Servos, M. R., Muir, D. C. G. & Webster, G. R. B. (1989). The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-p-dioxins. *Aquatic Toxicology, 14*, 169-184.

Simon, J. & Laginestra, E. (1997). *Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin Heliocidaris tuberculata.* (National Pulp Mills Research Program Technical Report 20). Canberra: CSIRO.

Skacel, A., Riha, V., Radclavsky, K. & Hlavata, M. (1993). The use of the Microtox system for evaluation of toxicity of wastes in Vitkovice Steelworks, Ostrave (Czechoslovakia)., *The Science of the Total Environment.* (Vol. Supplement 1993, pp. 195-205). Amsterdam.: Elsevier Science Publishers B.V.

Smith, B. (1995). *Tamar intertidal invertebrates*. Launceston: Queen Victoria Museum.

Smith, R. W. & Jenne, E. A. (1991). Recalculation, evaluation, and prediction of surface complexation constraints for metal adsorption on iron and manganese oxides. *Environmental Science & Technology, 25*, 525-531.

Stark, J. S. (1998). Heavy metal pollution and macrobenthic assemblages in soft sediments in two Sydney estuaries, Australia. *Marine Freshwater Research, 49*, 533-540.

Stauber, J. & Florence, T. M. (1985). Interactions of copper and manganese: a mechanism by which manganese alleviates copper toxicity to the marine diatom *Nitzchia closterium. Aquatic Toxicology, 7*, 241-254.

Stauber, J. L., Krassoi, R., Simon, J. & Laginestra, E. (1996). *Bioassay for testing sublethal toxicity in effluents, using gametes of sea urchin Heliocidaris tuberculata.* (natioanl Pulp Mills Research Program Technical Report No.19.). Canberra: CSIRO.

Stauber, J. L., Tsai, J., Vaughan, G. T., Peterson, S. M & Brockbank, C. I. (1994). *Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills* (National Pulp Mills Research Program Technical Report Series No.3): CSIRO Division of Coal and Energy Technology Centre for Advanced Analytical Chemistry.

Swartz, R. C., Ferraro, S. P., Lamberson, J. O., Cole, F. A., Ozretich, R. J., Boese, B. L., Schults, D. W., Behrenfeld, M. & Ankley, G. T. (1997). Photoactivation and toxicity of mixtures of polycyclic aromatic hydrocarbon compounds in marine sediment. *Environmental Toxicology and Chemistry., 16*(10), 2151-2157.

Talman, S., O'Connor, N., Zampatti, B. & Cannon, F. (1996). *Mount Lyell Remediation - Monitoring of benthic invertebrates in Macquarie Harbour, western Tasmania* (Supervising Scientist Report 115). Barton ACT: Department of Environment and Land Management.

Tamar River Master Planning Authority Report. (1990).

Tessier, A., Fortin, D. & Belzile, N. (1996). Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: narrowing the gap between field and laboratory measurements. *Geochimica et Cosmochimica Acta, 60*(387-404).

Thrower, S. J. & Eustace, I. J. (1973). Heavy metal accumulation in oysters grown in Tasmanian waters. *Food Technology in Australia, 25*, 546-553.

Troup, B. N., Bricker, O. P. & Bray, J. T. (1974). Oxidation effect on the analysis of Fe in the interstitial water of recent anoxic sediments. *Nature, 249*, 237-239.

Underwood, A.J. (1981). Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography and Marine Biology: Annual Review, 19*,513-605.

Watzin, M. C. & Roscigno, P. R. (1997). The effects of zinc contamination on the recruitment and early survival of benthic invertebrates in an estuary. *Marine Pollution Bulletin, 34*(6), 443-455.

Wildhaber, M. L. & Schmitt, C. J. (1998). Indicies of benthic community tolerance in contaminated Great Lakes sediments - relations woth sediment contaminant concentrations, sediment toxicity, and the Sediment QualityTriad. *Environmental Monitoring and Assessment, 49*(1), 23-49.

Williams, D. H. C. & Anderson, D. T. (1975). The reproductive system, embryonic development, larval development and metamorphosis of the sea urchin *Heliocidaris erythrogramma* (Val.) (Echiniodea: Echinometridae). *Australian Journal of Zoology, 23*, 371-403.

Wilson, J. G. & Jeffrey, D. W. (1994). Benthic biological pollution indices in estuaries. In J. M. Kramer (Ed.), *Biomonitoring of Coastal Waters and Estuaries.* (pp. 311-327). Boca Raton: CRC Press.

Winger, P. V. & Lasier, P. J. (1991). A vacuum-operated pore water extractor for estuarine and freshwater sediments. *Archives of Environmental Contamination and Toxicology, 21*, 321-324.

CHAPTER THREE

Shallow subtidal benthic macroinvertebrate assemblages associated with trace metal and organic pollution in two northern Tasmanian estuaries

J.A. Mondon[1], B.F. Nowak[1] and A.M.M. Richardson[2]

[1]School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Launceston, Tasmania 7250, Australia
[2]School of Zoology, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Sandy Bay, Tasmania 7000, Australia

Submitted for publication in *Environmental Toxicology & Chemistry*

ABSTRACT

A non-metric multidimensional scaling (MDS) ordination of community level data was performed as a complementary assessment to a sediment quality triad (SQT) evaluation of contaminated shallow subtidal sediments. Inference based on the SQT assessment of metal and organics contaminated shallow subtidal sediment indicated the likelihood of ecological stress associated with chemical toxicity. Use of multivariate multi-species analysis allowed detailed assessment of the putatively impacted benthic assemblages. Multi-dimensional scaling ordination and univariate analyses employing the PRIMER package identified significant differences between the patterns of distribution and abundances of benthic fauna from contaminated and non-contaminated estuaries. There was a significant correlation between patterns of assemblages and concentrations of trace metals, but granulometry and salinity were not linked to benthic assemblage patterns. Contaminated sediments were characterised by a greater abundance of nephtyids and callianassids, while non-contaminated sediments exhibited greater abundance of gastropods and polychaetes and were generally more diverse in terms of the number of species indicative of non-contaminated sediments.

Introduction

Ecotoxicological assessment of apparently polluted marine sediments requires the evaluation of contaminant effects on populations or communities under field conditions (Maund *et al.*, 1999), involving measurement of biological and chemical components (Wilson & Jeffrey, 1994). Comprehensive sediment quality assessment techniques such as Long & Chapman's (1985) sediment quality triad (SQT), involve concurrent measurement of sediment infauna, chemistry and toxicity data via field surveys, and toxicity tests of contaminated media. While the strength of the SQT analysis for retrospective assessment lies in its weight-of-evidence approach to evaluate and characterise potential risk to the aquatic ecosystem (Chapman, 1990; Chapman, 1996), limitations appear in the difficulty in detecting patterns in biotic assemblages which can be related to specific environmental variables or contaminant mixtures. Chemical and biotic components are generally presented as a single measure or index value (eg. sum of selected metals, and species diversity indices) which necessarily results in a loss of information from the complex field data set.

An alternative approach to the SQT is that of multivariate analysis of community-level data (Clarke, 1993). Application of a combination of multivariate classification and ordination techniques (such as non-metric multi-dimensional scaling (MDS) (Kruskal, 1964), combined with variations of Mantel permutation tests (Mantel, 1967), facilitate in the assessment of pollution-induced biological change at the population or community level (Clarke & Warwick, 1994). A key strength of Clarke's non-metric multivariate analysis of community-level data (collectively referred to in this paper as the MDS based approach), lies in its ability to analyse the community data set as a whole. Multivariate analyses of multispecies data are more likely to discriminate between sites than univariate summaries of the same data (Maund *et al.*, 1999). Although community structure analysis cannot provide definitive identification of cause and effect (Matthiessen *et al.*, 1995), the multivariate techniques presented in the PRIMER (Plymouth Routines in Multivariate Ecological Research) package (Clarke & Warwick, 1994) relate biotic effects in the field to all measured environmental variables, providing strong circumstantial evidence as to which

environmental variables, taken collectively, are most likely to be affecting community structure (Clarke, 1993; Clarke & Warwick, 1994).

A recent ecotoxicological assessment of contaminated shallow subtidal marine sediments (Mondon *et al.*, 1999), applied the SQT approach to determine sediment quality of putatively contaminated and non-contaminated regions within the lower reaches of two northern Tasmanian estuaries. Contaminated sites in the Tamar River estuary were compared with multiple reference sites (Underwood, 1989; Glasby, 1997) in the adjacent Port Sorell estuary. Port Sorell was chosen as a reference estuary based on its proximity and geochemical similarity to the Tamar River (Mondon, unpublished), and the absence of heavy industry. Both estuaries are permanently open to the sea, and experience two tidal cycles approximately every 24 hours. The lower reaches of each estuary exhibit fine sandy shallow subtidal substrates, with porewater salinity ranging between 28 ppt and 36 ppt (Mondon, unpublished). The region of greatest concern, Deceitful Cove on the Tamar River estuary, is an area previously exposed to high frequency, high intensity release of industrial smelting wastes which after more than two decades gradually decreased to lower frequency, lower intensity press (continous over a longer period of time) and intermittent pulse disturbances (D. Hassell, *pers. comm.*). Differential SQT responses (Chapman, 1990) and comparisons with sediment quality guidelines (SQGs) (Long *et al.*, 1995) indicated the strong likelihood of a difference or change in benthic community structure having occurred, due to toxicity associated with chemical contamination in the bay (Mondon *et al.*, 1999). Absence of *a priori* knowledge of the subtidal macro-invertebrate community precluded a definitive assessment as to whether an alteration in benthic community structure has occurred. The SQT was a post impact study, rather than a BACI (Before-After, Control-Impact) investigation (Green, 1979). As contamination of sediments had already occured, and prexisting baseline data was not available, the impact was inferred from spatial patterns among locations differing in degrees of imapct (Green, 1979). Consequently, it is unclear as to whether the benthic community structure present is representative of a chemical disturbance resulting in negligible change in the sediment biota, or disturbance causing either temporary, longer-term or permanent change (Skilleter,

1995). However, species diversity was shown to decrease with increasing chemical loading (based on principle components analysis of bulk chemical data).

The reduction of data to generate diversity indices for the purposes of the species abundance differential SQT analysis wastes much the community data, excluding the possibility of recognising assemblage patterns within the surveyed localities. Species predominantly responsible for determining the group assemblages were not readily identifiable from the diversity indices data. Additionally, associating macroinvertebrate assemblages to patterns in the physical and chemical environment in the SQT study was limited to linking measures of diversity with environmental variables, rather than community assemblages as a whole. The MDS based approach facilitates a detailed investigation of community patterns, the species responsible for them, and the environmental variables most likely to explain them, so it was appropriate to apply the MDS based framework to chemical and biota data from the SQT field survey.

In terms of the overall ecotoxicological assessment, the MDS based approach is not an alternative or stand alone series of analyses, but rather a complementary assessment to the SQT. The MDS based approach has been used in this study, in conjunction with the SQT, as the first stage in assessment of heavy metal pollution of shallow subtidal sediment in two northern Tasmanian estuaries. The aims were to establish whether the benthic macro-invertebrate assemblages at putatively polluted locations in the Tamar River estuary were different from assemblages found in "non-polluted" locations at Port Sorell, to determine which species were responsible for any differences between assemblages, and to determine whether a correlation existed between observed patterns in the biotic assemblages and measured environmental variables.

Sampling design and collection

Surface sediment samples were collected at four locations: Deceitful Cove (DC) and East Arm (EA) in the Tamar River estuary, Squeaking Point (SP) and North East Arm (NA) in the Port Sorell estuary (Figure 1). All locations were sampled twice during summer and winter (1996-1997) with a two week duration between collections. On each occasion four pooled samples from within a $1m^2$ quadrat were collected at four replicate sites, approximately 300 m apart, per location. Sampling was conducted at lowest low tide (this region experiences 2 low tides every 24 hours) and involved collection of sediments from approximately 0.5 m - 1 m water depth. The positions of sites within each location were marked using a series of visual fixes from the shore and an Eagle AccuNav Sport™ GPS (accuracy approx. 15-100 m).

Biota samples were collected using 7 cm diameter benthic core tubes inserted by hand to a depth of 10cm below the sediment water interface. Corers were chosen for sample collection to minimise loss of surface sediment due to bow wave generation (which occurs when benthic grabs and similar devices are used), and to minimise potential long term gross disturbance of the sediment. Whole sediment cores were placed on ice in a cooler during transportation to the laboratory where each sediment core was carefully sorted with 0.2 μm filtered seawater plus rose bengal, using a 1 mm Endecott sieve (Gray, 1981). Specimens collected were preserved in 70% alcohol and identified to the lowest practical taxonomic level. Most organisms were identified to at least genus level, but the majority of polychaete worms were identified to family level.

Four replicate cores from each site were collected for geochemical analysis of sediments. A full description of sample collection, storage and analytical procedure for salinity, ammonia, TOC, trace metals and organics, and granulometry is presented in Mondon *et al.*, (1999).

Macroinvertebrate analysis

Univariate, distributional and multivariate analysis of the macroinvertebrate assemblages associated with different sites was carried out using PRIMER (Plymouth Routines in Multivariate Ecological Research), a suite of programs developed for analysis of community abundance data by Plymouth Marine Laboratory, Plymouth (Clarke, 1993; Clarke & Warwick, 1994). A two-dimensional non-metric multidimensional scaling (MDS) ordination based on Bray-Curtis similarity matrices (Bray & Curtis, 1957) of fauna in all samples was performed to display the relationships among samples of organisms. A minimum of 10 iterations were calculated per MDS ordination. Stress values of < 0.1 were accepted as providing a good to excellent representation of the overall stucture (Clarke & Warwick, 1994). Rare species, whose occurrence at a particular site may largely be due to chance, were excluded by removal of species accounting for <3% of the total abundance in any one sample (Clarke & Warwick, 1994). Statistically significant differences between faunal assemblages at different locations were determined using the multivariate ANOSIM (analysis of similarities) module. ANOSIM generates r-statistics, measures of discrimination based on Bray-Curtis similarity matrices (Clarke & Green, 1988). Additionally, univariate SIMPER analysis (similarity % procedure) was used to identify the taxa contributing most to differences between contaminated and reference locations, and to identify species that typified each location and estuary (Clarke, 1993).

Environmental variables were superimposed on biotic ordinations linking community assemblages to environmental variables. Analysis to determine the environmental variables that best explain the biotic ordination was performed using the BIOENV procedure, where associations between species abundance data and log transformed environmental variables were measured by weighted Spearman rank correlation between biotic and abiotic similarity matrices (Clarke & Warwick, 1994). Collinearity between log transformed environmental variables was determined using Pearson's correlation coefficient (JMP®, SAS Institute Inc.). Variables with a mutual correlation averaging greater than 0.9 were reduced to a single representative subset prior to the BIOENV procedure being performed.

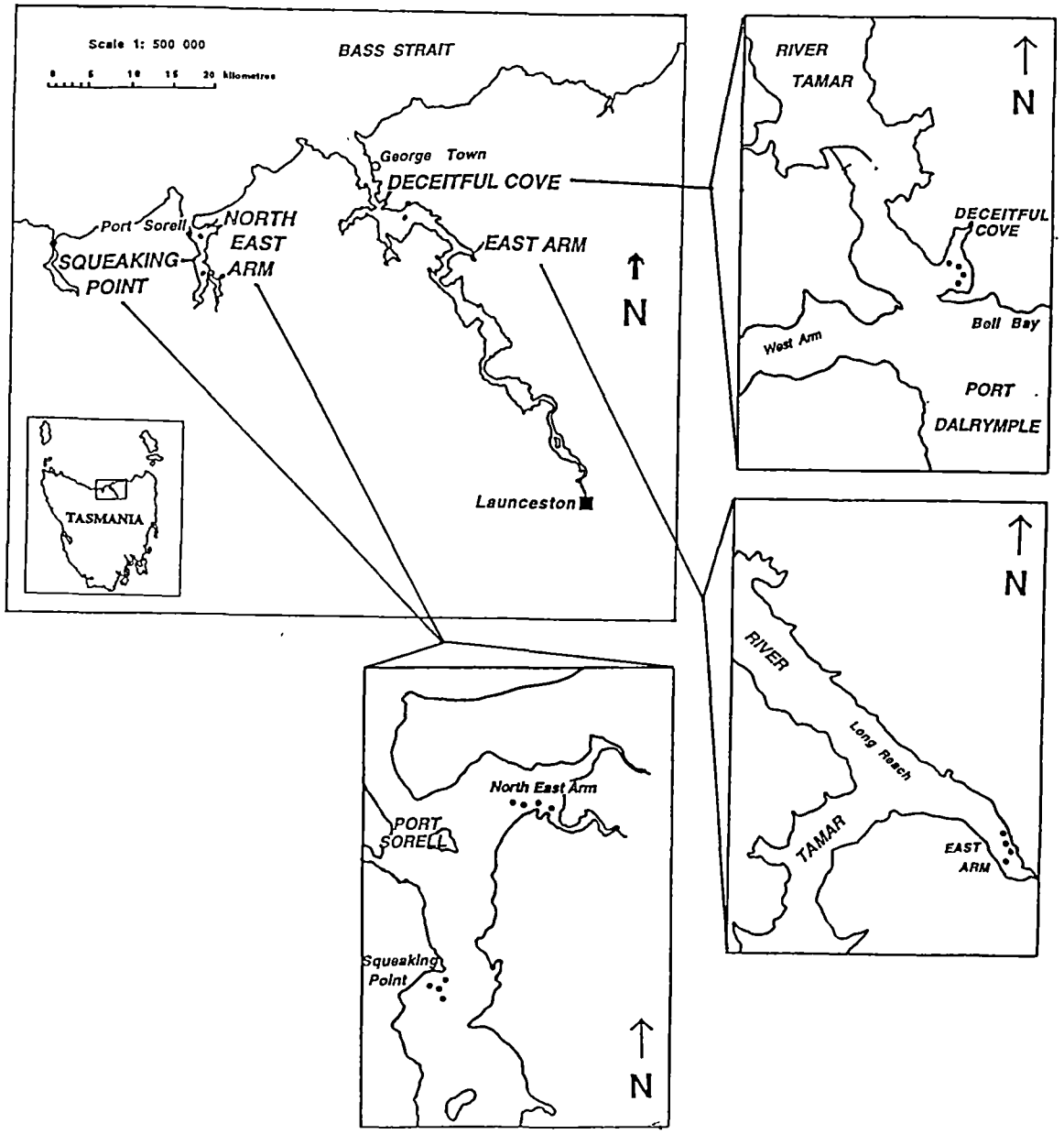


Figure 1. Location of research area: Deceitful Cove and East Arm in the Tamar River estuary; Squeaking Point and North east Arm in the Port Sorell estuary. Black circles represent sampling sites.

Results

A total of 2113 individuals representing 69 taxa from 7 phyla were collected from shallow subtidal sediments in the lower Tamar River and Port Sorell estuaries. The mean (S.E.) number of taxa /450m^3 sediment varied markedly between survey locations: 20.5±1.75 (Deceitful Cove), 24.0±1.58 (East Arm), 29.5±0.87 (Squeaking Point) and 38.7±0.63 (North East Arm).

Hierarchical clustering (Bray-Curtis similiarity) of macroinvertebrate data produced four groups with similarity between 45% and 68% (Figure 2). Each group corresponded to one of the four survey locations. Community assemblages within Deceitful Cove are closest to those in East Arm. Conversely, community structure within Squeaking Point is closer to North East Arm than to either Tamar River estuary locations. Whilst the clusters imply different characteristic patterns of abundance found consistently within each location, clustering does not imply the groups are primarily comprised of species unique to each group.

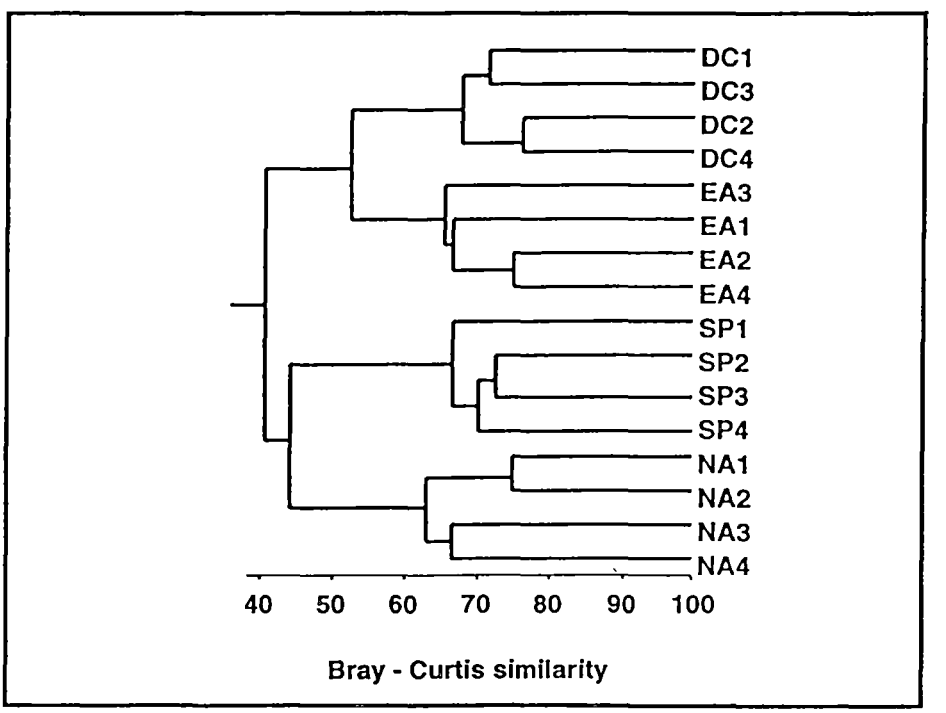


Figure 2. Dendrogram showing results of hierachical clustering of the 16 field survey sites, using group-average linking of Bray-Curtis similarities calculated on species abundance data: DC = Deceitful Cove; EA = East Arm; SP = Squeaking Point; NA = North East Arm

Superimposing the groupings from the cluster analysis on an MDS ordination of species abundances based on Bray-Curtis similarity measures shows consistency between both procedures (Figure 3). Clusters are sharply defined with little distortion. Each site is clearly identified by membership in one of four locality groups, showing distinct differences in community assemblage within and between estuaries. The ordination stress is relatively low (0.09), indicating the 2 dimensional plot is an accurate representation of the sample relationships. Analysis of similarity indicates that the four groups are significantly different from each other (ANOSIM: global r=0.985, $p< 0.05$).

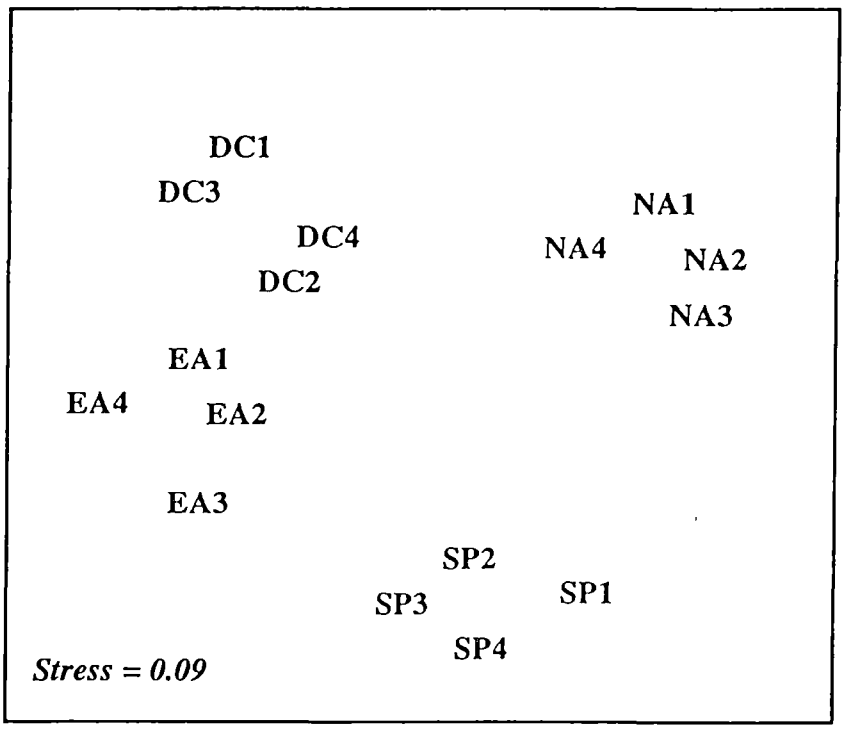


Figure 3. MDS ordination of 16 survey sites based on non-transformed Bray-Curtis similarities calculated on non-transformed species abundance data: DC = Deceitful Cove; EA = East Arm; SP = Squeaking Point; NA = North East Arm

Similarity analysis (SIMPER) identified a number of likely discriminating species responsible for determining the observed group clusters, with several species responsible for determining similarity within locations. The polychaete *Nephtys australiensis* contributed most to similarities (54% similarity) within the Tamar River, whereas *Nephtys australiensis*, accounted 16% to the similarities at Port Sorell (Table 1).

Table 1. Top five taxon contributing most to similarities within pooled locations (Bray-Curtis, ranked in order of importance).

Location	Average similarity	Species	Percent contribution	Cumulative percent
Tamar River	61.09	*Nephtys australiensis*	54.33	54.33
		Tellina deltoidalis	7.77	62.11
(Deceitful Cove		*Nassarius pauperatus*	6.74	68.84
+ East Arm)		*Lumbrinereis sp.*	4.28	73.12
		Macrophthalmus latifrons	3.24	76.36
Port Sorell	62.11	*Mysella donacformis*	19.05	19.05
		Nephtys australiensis	16.14	35.19
(North East Arm		*Katelysia scalarina*	12.01	47.2
+ Squeaking Point)		*Magleona sp.*	7.24	54.44
		Lumbrinereis sp	6.04	60.48

Several species occurred consistently within locations and can be considered to be typical or characteristic of each location. The number of "typical" species is proportionally higher at Port Sorell (Table 2).

Table 2. Species indicative of each location based on discrimination ratios (s_i,/SD(s_i)>3, ranked in order of decreasing abundance).

Deceitful Cove	East Arm	Squeaking Point	North East Arm
Nephtys australiensis	*Nephtys australiensis*	*Mysella donacformis*	*Nephtys australiensis*
Katelysia scalarina	*Nassarius pauperata*	*Nephtys australiensis*	*Katelysia scalarina*
Lumbrineris sp.	*Tellina deltoidalis*	*Katelysia rhytiphora*	*Mysella donacformis*
Callianasa ceramica	*Magleona sp.*	*Katelysia scalarina*	*Magleona sp.*
Tellina deltoidalis	Orbinidae sp. 2	*Nassarius pyrrhus*	*Lumbrineris sp.*
Nassarius pyrrhus	*Heteromastus sp.*	*Nassarius pauperata*	*Tellina deltoidalis*
	Mysella donacformis	*Magleona sp.*	*Nassarius pyrrhus*
	Terebellides sp	*Lumbrineris sp*	*Nassarius pauperata*
	Tellina marginata	*Heteromastus sp.*	*Birubus sp.*
		Orbinidae sp. 2	*Scoloplos simplex*
		Tellina deltoidalis	*Callianasa ceramica*
		Birubus sp.	*Lasaea australis*
		Callianasa ceramica	*Eumarcia fumigata*
		Heloecius cordiformis	Cirratulidae
			Turbonilla fus
			Macrophthalmus latifrons
			Phyllodocidae
			Laternula tasmanica
			Maldanidae

Several species contribute to the dissimilarity between the two estuaries (Table 3). Just over 90% of the contribution to dissimilarity between the Tamar River and Port Sorell is accounted for by the first 35 species, with over 50% accounted for by the first six species. Approximately 90 % of the difference between Deceitful Cove and East Arm can be attributed to the first 31 species, with over 50% from first 8 species. Likewise 90% of the difference between Squeaking Point and North East Arm can be attributed to the first 39 species, with over 50% accounted for by the first 11 species.

Table 3. Species contributing to up to 50% dissimilarity between locations (Bray-Curtis dissimilarity, ranked in order of importance).

Dissimilarity between locations	Average dissimilarity	Taxon id.	Percent contribution	Cumulative percent
Tamar River and Port Sorell	58.03	*Nephtys australiensis*	21.07	21.07
		Mysella donacformis	11.55	32.62
		Katelysia scalarina	6.65	39.27
		Nassarius pauperatus	4.61	43.88
		Magelona sp.	4.02	47.9
		Katelysia rhytiphora	3.7	51.59
Deceitful Cove and East Arm	45.17	*Nassarius pauperatus*	10.92	10.92
		Katelysia scalarina	10.12	21.04
		Callianassa ceramica	8.25	29.29
		Nephtys australiensis	7.33	36.62
		Lumbrinereis sp.	6.23	42.85
		Nassarius pyrrhu	3.36	46.21
		Tellina deltoidalis	3.18	49.39
		Maldanidae	3.01	52.39
Deceitful Cove and Squeaking Point	56.50	*Nephtys australiensis*	23.38	23.38
		Mysella donacformis	15.61	38.99
		Callianassa ceramica	6.32	45.31
		Katelysia rhytiphora	5.83	51.14
		Magelona sp.	3.98	55.13
Deceitful Cove and North East Arm	54.19	*Nephtys australiensis*	24.83	24.83
		Mysella donacformis	10.13	34.96
		Katelysia scalarina	6.71	41.67
		Callianassa ceramica	6.07	47.75
		Magelona sp.	5.22	52.97

The species most likely to be good discriminators between sets of groups are indicated in Table 4. The majority of species primarily responsible for discriminating between locations are determined by differences in average abundance. Very few discriminator

species were unique to a particular location. Some species were present at only one location or within one estuary: 28% of species were found in one location, 7.25% were found in one estuary. With the exception of one nemertean (unidentified) and the mollusc *Turbonilla (Pyryiseus) fus*, all "unique" species were "rare', (accounting for <3% of the total abundance per sample) and so their detection was largely due to chance.

Table 4. Species responsible for discriminating between localities (Bray-Curtis dissimilarity, ranked in order of importance). Species likely to be good discriminators of groups 1 and 2 are indicated by an asterisk.

Species	Group 1 Mean abundance	Group 2 Mean abundance	Ratio $s_i/SD(s_i)>2$	Percent contribution to dissimilarity
	Tamar River	Port Sorell		
Nephtys australiensis	19.75	33.38	4.74	21.07*
Mysella donacformis	24.13	1.00	2.98	11.55*
	Deceitful Cove	East Arm		
Nassarius pauperatus	2.75	11.25	2.23	10.92*
Katelysia scalarina	9.75	0.5	2.22	10.12*
Callianassa ceramica	8.0	0.75	2.04	8.25*
Nephtys australiensis	37.5	29.25	2.64	7.33*
Lumbrinereis sp.	7.5	1.5	2.24	6.23*
Orbinidae	0.5	2.5	2.70	2.72
Mysella donacformis	0.25	1.75	2.49	2.00
Tellina marginata	0.25	1.0	2.22	1.03
	Deceitful Cove	Squeaking Point		
Nephtys australiensis	37.50	16.25	6.98	23.37*
Mysella donacformis	0.25	18.75	8.01	12.02*
Callianassa ceramica	8.0	1.75	2.03	6.35*
Katelysia rhytiphora	1.5	11.0	4.19	5.98*
Birubus sp.	0.25	2.0	2.19	1.09
	Deceitful Cove	North East Arm		
Nephtys australiensis	37.5	23.25	9.05	24.73*
Mysella donacformis	0.25	23.75	2.87	10.13*
Scoloplos simplex	0.0	6.25	2.01	2.85
Birubus sp.	0.25	6.25	2.12	2.5
Nemertea	0.0	2.75	2.15	1.27
Eumarcia fumigata	0.75	2.0	2.44	1.06
Lasaea australis	0.0	2.0	10.73	0.9
Cirratulidae	0.0	1.75	2.35	0.76
Laturnula tasmanica	0.5	1.25	2.12	0.70
Maldanidae	0.5	1.25	2.12	0.70
Phyllodocidae	0.25	1.5	2.49	0.65

Table 5. Relative abundance of discriminator species at Deceitful Cove, East Arm, Squeaking Point and North East Arm.

Group 1 vs Group 2	Expected increase in species abundance in Group1 relative to abundance in Group 2	Expected decrease in species abundance in Group 1 relative to abundance in Group 2
Tamar River vs Port Sorell	*Nephtys australiensis - 69%*	*Birubus sp.- 96.5%*
Deceitful Cove vs East Arm	*Nephtys australiensis - 28%* *Lumbrinereis sp.- 400%* *Katelysia scalarina - 1850%*	*Nassarius pauperata - 76%* Orbinidae sp. 2 - 80% *Tellina marginata - 75%*
Deceitful Cove vs Squeaking Pt.	*Nephtys australiensis - 131%* *Callianasa ceramica - 357%*	*Mysella donacformis -99%* *Katelysia rhytiphora - 86%* *Birubus sp.- 87.5%*
Deceitful Cove vs North East Arm	*Nephtys australiensis - 61%*	*Mysella donacformis -100%* *Scoloplos simplex - 100%* *Birubus sp.- 96%* Nemertea sp.3 - *100%* *Eumarcia fumigata - 62.5%* *Lasaea australis - 100%* Cirratulidae - *100%* Maldanidae - *100%* *Laternula tasmanica - 60%* Phyllodocidae - *83%*

Nephtys australiensis and *Mysella donacformis* are principally responsible for discriminating between the Port Sorell and Tamar River benthic communities. *Nephtys australiensis* exhibits a higher discriminatory power than *Mysella donacformis* yet the relative abundance is the opposite: *Nephtys australiensis* is approximately 1.7 times more abundant in the Tamar River estuary, whereas *Mysella donacformis* is approximately 24 times more abundant at Port Sorell. A similar pattern emerges when narrowing the dissimilarity analysis to a comparison between Deceitful Cove and Port Sorell locations. The discriminatory ratio for *Nephtys australiensis* ranges between 6.98 (Squeaking Point) and 9.05 (North East Arm). In both cases the abundance of *Nephtys australiensis* is consistently higher at Deceitful Cove (Table 4). Estimated relative abundance of discriminator species suggests a smaller assemblage of indicator species at relatively higher abundances in the Tamar River estuary than at Port Sorell localites (Table 5). Those species occurring consistently within locations and considered as contributing significantly to differences between locations (ie. with a contribution greater than 4% overall, (as indicated

by an asterisk in Table 4) can be used to differentiate sites based on the relative abundance of discriminator species (Clarke & Warwick, 1994).

Correlation of the biotic pattern with contaminant variables is illustrated by the superimposition of contaminants. Figure 4 (d - i) displays the values of contaminants in the sediment as circles of varying diameter confirming the main axis of the biotic MDS ordination as one of increasing contamination. Several variables were collinear: Al and %TOC; Cr and Fe; Zn, Cd, Ni and Ag; Pb and Mg (Table 6).

Table 6. Collinear variables determined by Pearson's correlation coefficient.

Environmental Variable	Collinear Variable	Correlation	Probability
Al	%TOC	0.9013	<0.0001
Cr	Fe	0.9826	<0.0001
Zn	Cd	0.9739	<0.0001
Zn	Ni	0.9764	<0.0001
Zn	Ag	0.9045	<0.0001
Pb	Mn	0.9637	<0.0001

Table 7. Combinations of 8 environmental variables, taken *k* at a time, giving the largest weighted Spearman rank correlation between environmental and biotic similarity matrices: bold type indicates best possible result.

k	Best variable combination (p_w)
1	Al (0.719) ; Cu (0.635)
2	Cr, Zn (0.716) ; Al, Cr (0.697)
3	**Al, Cr, Cu (0.725) ; Al, Pb, Cr (0.722)**
4	**Al, Cr, Cu, Zn (0.721)** ; Al, Pb, Cr, Cu (0.702)
5	Al, Cr, Cu, Zn, Pb (0.704) ; Al, Pb, Cr, Cu, PAHs (0.692)
6	Al, Cr, Cu, Zn, Pb, PAHs (0.679) ; Al, Cr, Cu, Zn, PAHs, %mud (0.653)
7	Al, Pb, Cr, Cu, Zn, PAHs, salinity (0.625) ; Al Cr, Cu, Zn, Pb, PAHs, %mud (0.601)
8	Al, Pb, Cr, Cu, Zn, PAHs, salinity, %mud (0.565)

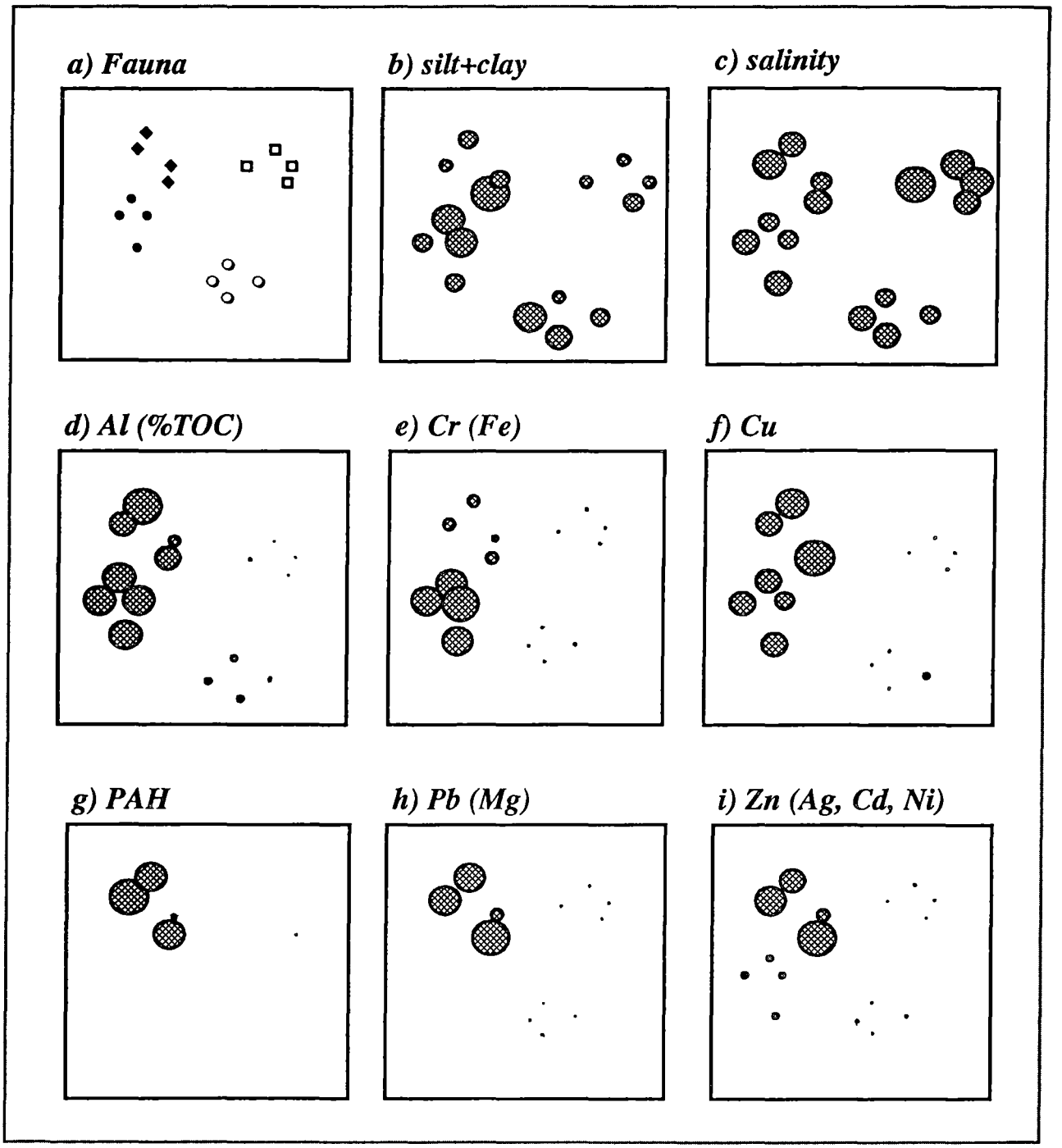


Figure 4. **Environmental variables (*b-i*) superimposed on MDS of Bray-Curtis similarities between sites, based on pooled species samples (Stress = 0.09); diameter of circles scaled to represent actual value. Collinear variables represented in brackets. (◆) Deceitful Cove; (●) East Arm; (○) Squeaking Point; (□) North East Arm.**

Matching of biotic patterns to environmental variables indicates that several combinations of variables best "explain" the biotic distribution: Al+Cr+Cu (ρ_w=0.725), Al+Pb+Cr (ρ_w =0.722), Al+Cr+Cu+Zn (ρ_w =0.721 (Table 7). Aluminium, Cr and Zn act as proxy for other potentially causal collinear variables (Table 6) not included in the correlation coefficient to minimise potentially confounding effects.

The abiotic ordination plot for the best combination at increasing levels of complexity (i.e. at increasing numbers of variables (k) at a time), demonstrates a reasonable degree of concordance between biotic and abiotic plots (Figure 5). Figures 5 d, e & f represent the most accurate environmental combinations that best explain the community pattern. The greatest degree of concordance stems from the matching structure of Deceitful Cove and Squeaking Point in Figures 5 e & f (Al+Cr+Cu and Al+Cr+Cu+Zn respectively). East Arm, North East Arm and Squeaking Point in Figure 5d (Al+Pb+Cr) appear less distinguishable, with each group showing reduced spacing within the groups than in the biotic plot. The overall fit deteriorates with the introduction of PAHs, particularly with respect to the close proximity of Port Sorell and North East Arm groupings (Figure 5 h). The ordination pattern remains relatively unchanged with the introduction of salinity and %silt+clay (Figure 5 i & j).

Polychaetes are less abundant at Deccitful Covc relative to other locations despite the high abundance of *Nephtys australiensis.* Conversely, decapod numbers are higher at Deceitful Cove relative to other locations, most probably due to the high abundance of the ghost shrimp *Callianassa sp.* (Figure 6). Overall, gastropods appear to be less abundant in contaminated sediments, whereas there is no clearly identifiable pattern between contaminated and non-contaminated sediments with respect to bivalve abundance (Figure 6).

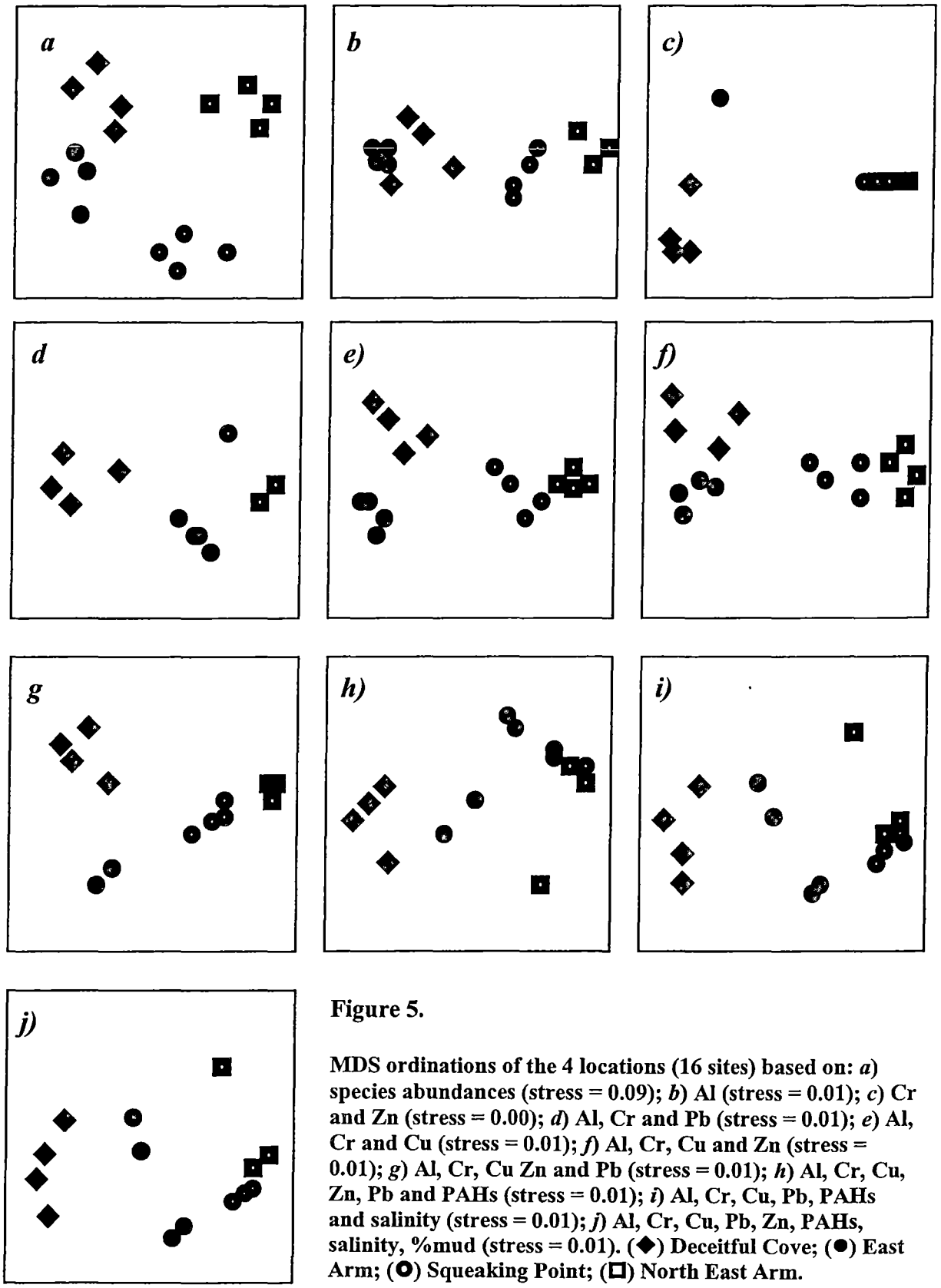


Figure 5.

MDS ordinations of the 4 locations (16 sites) based on: *a*) species abundances (stress = 0.09); *b*) Al (stress = 0.01); *c*) Cr and Zn (stress = 0.00); *d*) Al, Cr and Pb (stress = 0.01); *e*) Al, Cr and Cu (stress = 0.01); *f*) Al, Cr, Cu and Zn (stress = 0.01); *g*) Al, Cr, Cu Zn and Pb (stress = 0.01); *h*) Al, Cr, Cu, Zn, Pb and PAHs (stress = 0.01); *i*) Al, Cr, Cu, Pb, PAHs and salinity (stress = 0.01); *j*) Al, Cr, Cu, Pb, Zn, PAHs, salinity, %mud (stress = 0.01). (◆) Deceitful Cove; (●) East Arm; (O) Squeaking Point; (□) North East Arm.

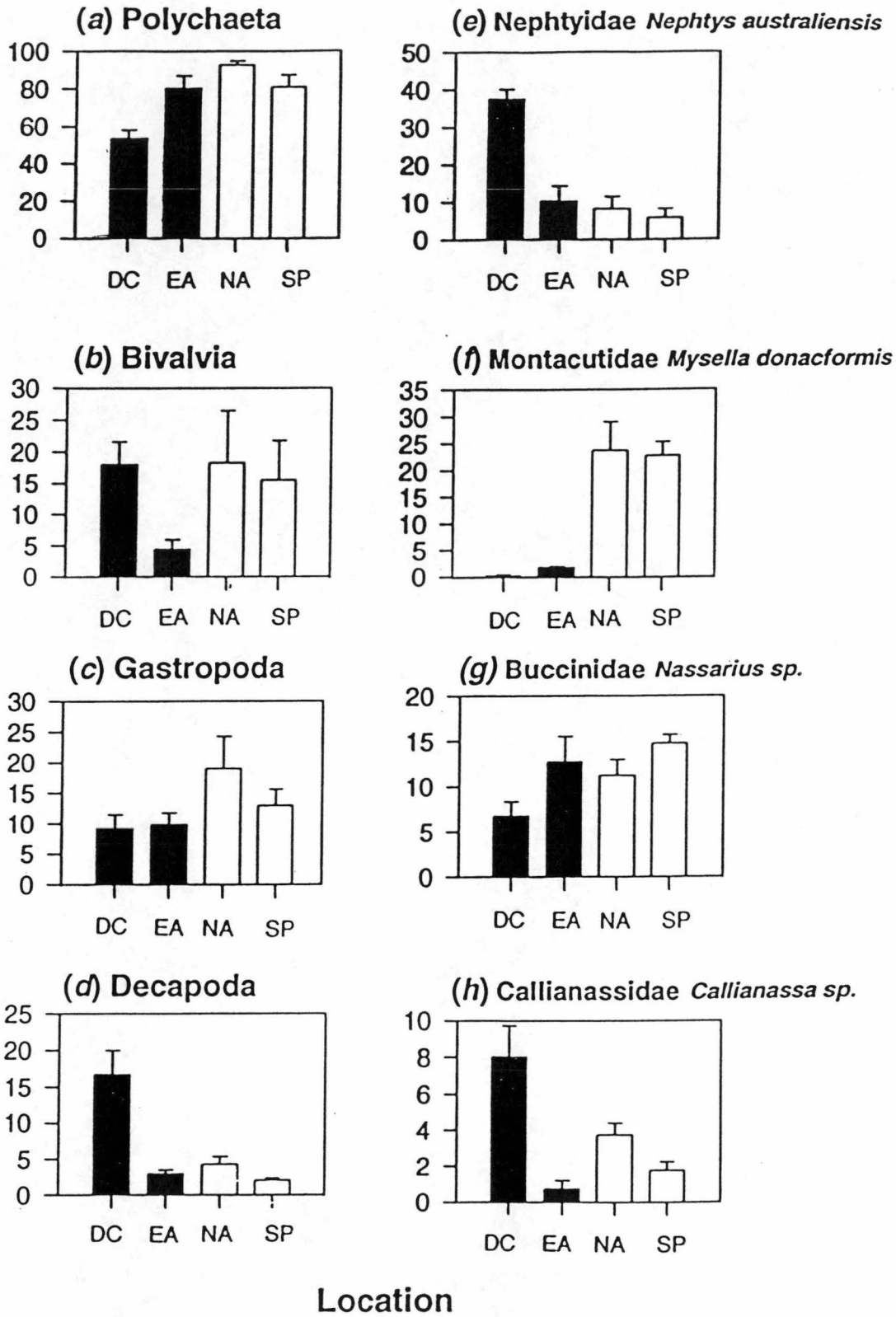


Figure 6. **Mean total abundance (± S.E.) for selected taxa at each location. Clear bars indicate reference locations, shaded bars contaminated locations. n = 128. DC = Deceitful Cove; EA = East Arm; SP = Squeaking Point; NA = North East Arm**

Discussion

Variation in benthic macroinvertebrate assemblages was clearly evident between polluted and non-polluted locations. A significant correlation existed between metal concentrations and assemblage patterns, supporting the hypothesis that biotic assemblages of the Tamar River estuary are associated with predominantly trace metal contamination. The biotic patterns of macroinvertebrate distribution were strongly related to the sediment concentrations of Al, Cr, Cu, Pb and Zn. However, collinear variables, Ag, Cd, Fe, Mg, Ni, and %TOC, could also correlate well with the biotic patterns observed.

Considering the potential ecotoxicological impact associated with long term metals exposure in sediments, the likelihood of benthic community recovery is generally limited in the case of prolonged release of contaminants (Gray, 1981), and may result in permanent change in the nature and composition of the benthic communities present (Glasby & Underwood, 1996). However, the results suggest the benthic macroinvertebrate populations at Deceitful Cove exhibited considerable inertia towards contaminant exposure (Underwood, 1989). It is possible that potential tolerance to contamination at Deceitful Cove is attributable to long term exposure. Compensatory processes facilitating resilience to contaminants in individuals (Depledge, 1989), would enhance the survivorship of those individuals and their offspring over the period of contamination of sediments conditions (Luoma, 1996). The current populations of species with non-planktonic larval stages present at Deceitful Cove are therefore likely to be the descendants of individuals which were least sensitive to the chemically modified environment.

Adverse effects of pollution, manifested as depauperate populations or species absence (Skilleter, 1995; Luoma, 1996) were not evident in this study. Additionally, the trend towards an increase in abundance of opportunistic species commonly detected in stressed or polluted marine sediments (Pearson & Rosenberg, 1978; Carr *et al.*, 1996; Luoma, 1996; Ward & Hutchings, 1996; Stark, 1998a) was also not apparent. Opportunistic polychaetes from the families Spionidae, Capitellidae and Nereidae, indicative of polluted estuaries in the Sydney region (Stark, 1998a), were either "rare" or in low abundance at Deceitful Cove. Likewise, polychaetes from the family Glyceridae, associated with trace metal

contaminated sediments of Spencer Gulf, South Australia, were not abundant in the contaminated sediments of the Tamar estuary. *Heteromastus sp.*, from the family Capitellidae, was only indicative of East Arm sediments where relatively moderate levels of metals enrichment exist.

The lower overall abundance of polychaetes at Deceitful Cove, relative to other locations, represents a potential sensitivity to trace metal contamination by polychaete taxa as a whole (McClusky & Phillips, 1975). Species exhibiting reduced abundance in contaminated sediments may be associated with reduced recruitment (Luoma & Ho, 1993), particularly where disturbance takes place over a long period of time and variation in rates of breeding, settlement of larvae, immigration and emigration may be instrumental in directing change (Morrisey, 1995). Individuals inhabiting the contaminated sites may also be phenotypically adapted to a less predictable, dynamic environment compared to individuals of the same species inhabiting non-contaminated subtidal sediments (Depledge, 1990). A combined field survey and experimental study found populations of several families of polychaetes adapted to highly elevated metal concentrations in the Sydney region estuaries (Stark, 1998a; Stark, 1998b). Very little of the biology of taxa present at Deceitful Cove is known, however, of the six species identified as indicative of Deceitful Cove, the gastropod *Nassarius (Zeuxis) pyrrhus* lay eggs which are thought to develop crawling young (Smith, 1995). This results in a localised dispersion of juveniles and allows the potential development of genetically adapted tolerance within the population.

The remaining indicator species, the ghost shrimp *Callianassa ceramica*, polychaetes *Nephytys australiensis* and *Lumbrineris sp.*, and bivalves *Katelysia scalarina* and *Tellina deltoidalis* exhibit broadcast dispersion of pelagic larvae. As the geographical scale of pollution within Deceitful Cove is likely to be smaller than the geographical distribution of individual pelagic larvae, the local population of individuals within the bay is likely to be recruiting from less contaminated habitats. Future studies may be able to determine the taxonomic selectivity of contaminated sediments on larval recruitment, and the physiological performance of species tolerant to the predominantly metal-contaminated conditions.

Very few species (less than 4% overall) were characteristic of only one locality, and none of these contributed markedly to discriminating between localities. In the absence of elevated abundance of known opportunistic species, difference in the relative abundance of discriminator species between contaminated and non-contaminated localities provides a potential indicator of disturbance, given that observed differences in biotic assemblages were not linked to sediment granulometry, salinity or ammonia. The abundances of several species were identified as being indicative of contaminated sediments, and the majority of these exhibited a wide distribution across contaminated and non-contaminated sites. *Nephytys australiensis*, the strongest discriminator and most abundant polychaete at Deceitful Cove, is found in a range of habitats and different Tasmanian estuaries (Edgar *et al.*, 1999). *Mysella donacformis*, the strongest discriminator at Port Sorell localities, was present in the Tamar River estuary. Future studies may be able to investigate the potential "indicator" species from contaminated and non-contaminated areas to determine the spatial extent of ecological effects and to monitor contaminant effects over time.

The ecological significance of lower numbers of individuals and taxa present within macro-invertebrate populations at Deceitful Cove, relative to non-contaminated sites, is unknown. Benthic organisms contribute significantly to ecosystem health by way of regulation of nitrogen, carbon and sulphur cycling, oxygenation of the substrate, pollutant distribution and fate, secondary production and transport, and stability of sediments (Snelgrove *et al.*, 1997). Benthic organisms already stressed by metal contaminants may not be tolerant of further disturbances (Peterson & Black, 1988). Additional chemical and physical disturbances might elicit a reduction in the number of surficial sediment dwelling organisms, resulting in a decrease in bioturbation (Carr *et al.*, 1996). Elevated anoxic conditions are then likely to occur due to a build up of ammonia and sulphides in sediments which are normally flushed and oxidised by bioturbation (Huttel, 1990; Carr *et al.*, 1996).

The multivariate analysis indicates that the impact on the macro-invertebrate community assemblage *in situ* may not be as bad as predicted by the direct measurements of pollutants and results of laboratory bioassays for Deceitful Cove sediments (Mondon *et al.*, 1999).

Stronger differences between the contaminated and reference sites were expected based on the premise that several SQG values were exceeded at Deceitful Cove. Additionally, the differential SQT assessment, which used species diversity indices, indicated the likelihood of a stressed environment at best, or at worst, environmental degradation having taken place. Other multivariate analyses of contaminated communities have highlighted the sensitivity of multivariate analyses over univariate methods for detecting impact on communitiy structure (Clarke & Warwick, 1994). Gray *et al.*'s (1990) multivariate analyses of macrobenthic communities in the region of the Norwegian Ekofisk oil field (North Sea), for example, indicated a distinct change in community pattern was clearly evident with increasing distance from the disturbance site that was not detectible by diversity indices. Considerable change to community structure involving increased abundance of opportunistic species was also detected only under the most severe pollution conditions.

Whilst a definitive assessment regarding change in the sediment biota is not clear, the MDS based approach has indicated that Deceitful Cove exhibits a different community structure relative to less contaminated and non-contaminated sites. There is strong evidence to suggest that multiple trace metals are linked to macroinvertebrate community structure, discounting the likelihood of a single causal variable. Several species have been identified as discriminator species tolerant of elevated metal concentrations, and warrant further investigation with respect to their use as indicator species for assessment and monitoring of contaminated sediments. This study illustrates the value of utilising the SQT and MDS based approach as complementary sediment assessments. The SQT evaluated and characterised the potential risk of contaminated sediments to the aquatic ecosystem based on weight of evidence. Multivariate analyses of multispecies assemblages identified significant differences between benthic assemblages the contaminants most likely to be responsible and the species most tolerant to the contaminated conditions. To establish the presence of pollution-caused degradation of the benthos, a link between chemistry and toxicity must be established, in addition to a link between toxicity and benthic index values. The findings of the study also warrant further investigation into the use of the discriminator species identified as indicators of sediment quality. Additionally, experimental

investigation aimed at establishing causal effects of trace metal pollution to alteration in subtidal benthic community structure is necessary.

Acknowledgements

This study was funded by the Australian Research Council, TEMCO and Comalco Aluminium (Bell Bay) Limited. The authors thank Dr Graham Edgar, School of Zoology, University of Tasmania, and Dr Brian Smith, Queen Victoria Museum, Launceston, Tasmania, for assistance with macroinvertebrate identification; Mr Jon Bryan, SeaNature Photography, and, Ms Susan Duda, Ms Marianne Helders and Mr Rob Gurney, School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, for their technical assistence in the field. Trace metal analyses of sediments were performed by Dr Ashley Townsend, Central Science Laboratory, University of Tasmania. Organics analysis of sediments were performed by Australian Environmental Laboratories, Hawthorn, Victoria.

References

Bray, J. R. & Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs, 27*, 325-349.

Carr, R. S., Chapman, D. C., Howard, C. L. & Biedenbach, J. M. (1996). Sediment Quality Triad assessment survey of the Galveston Bay, Texas system. *Ecotoxicology, 5*(6), 341-364.

Chapman, P. M. (1990). The Sediment Quality Triad approach to determining pollution-induced degradation. *Science of the Total Environment, 97/98*, 815-825.

Chapman, P. M. (1996). Presentation and interpretation of sediment quality triad data. *Ecotoxicology, 5*, 1-13.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology, 18*, 117-143.

Clarke, K. R. & Green, R. H. (1988). Statistical design and analysis for a "biological effects" study. *Marine Ecolology Progress Series, 46*, 213-226.

Clarke, K. R & Warwick, R. M. (1994). *Change in marine communities: an approach to statistical analysis and interpretation.* Plymouth: Plymouth Marine Laboratory.

Depledge, M. (1989). The rational basis for detection of the early effects of marine pollutants using physiological indicators. *Ambio, 18*, 301-302.

Depledge, M. H. (1990). New approaches in ecotoxicology: can inter-individulal physiological variability be used as a tool to investigate pollution effects? *Ambio, 19*, 251-252.

Edgar, G. J., Barrett, N. S., Graddon, D. J. & Last, P. R. (1999). The conservation significance of estuaries: a classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. *Biological Conservation (In press).*

Glasby, T. M. & Underwood, A. J. (1996). Sampling to differentiate between pulse and press perturbations. *Environmental Monitoring & Assessment, 42*, 241-252.

Glasby, T. M. (1997). Analysing data from post-impact studies using asymmetrical analyses of variance: A case study of epibiota on marinas. *Australian Journal of Ecology, 22*, 448-459.

Gray, J. S. (1981). *The ecology of marine sediments.* Oxford: Cambridge University Press.

Gray, J. S., Clarke, K. R., Warwick, R. M. & Hobbs, G. (1990). Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. *Marine Ecology Progress Series, 66*, 285-299.

Green, R. H. (1979). *Sampling design and statistical methods for environmental biologists.* Chichester: Wiley Interscience.

Huttel, M. (1990). Influence of the lugworm Arenicola marine on porewater nutrient profiles of sandflat sediments. *Marine Ecology Progress Series, 62*, 241-248.

Kruskal, J. B. (1964). Multidimensional scaling by optimizing goodness of fit to a non-metric hypothesis. *Psychometrica, 29*, 1-27.

Long, E. R. & Chapman, P. M. (1985). A Sediment Quality Triad: Measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Marine Pollution Bulletin, 16*(10), 405-415.

Long, E. R., MacDonald, D. D., Smith, S. L. & Calder, F. D. (1995). Incidence of adverse effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management., 19*, 81-97.

Luoma, S. N. (1996). The Developing framework if marine ecotoxicology: Pollutants as a variable in marine ecosystems? *Journal of Experimental Marine Biology and Ecology, 200*, 29-55.

Luoma, S. N. & Ho, K. T. (1993). Appropriate uses of marine and estuarine sediment bioassays. In P. Calow (Ed.), *Handbook of ecotoxicology* (Vol. 2, pp. 193-226). London: Blackwell Scientific Publications.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research, 27*, 209-220.

Matthiessen, P., Blackburn, M., Kirby, M., Law, R., Sheahan, D. & Thain, J. (1995). Toxicity of estuarine waters and sediments resulting from industrial chemicals. *Marine Environmental Management Review of 1994 and Future Trends, 2*(4), 23-26.

Maund, S., Chapman, P., Kedwards, T., Tattersfield, L., Matthiessen, P., Warwick, R. & Smith, E. (1999). Application of multivariate statistics to ecotoxicological field studies. *Environmental Toxicology and Chemistry, 18*(2), 111-112.

McClusky, D. S. & Phillips, C. N. K. (1975). Some effects of copper in the polychaete *Phlyllodoce maculata*. *Estuarine Coastal & Marine Science, 3*, 103-108.

Mondon, J., Richardson, A. M. M. & Nowak, B. F. (1999). Sediment Quality Triad assessment of trace metals and organic contamination of shallow subtidal marine sediments near a ferro-manganese smelter and aluminium refining plant. *Under review.*

Morrisey, D. (1995). Estuaries. In A. J. Underwood & M. G. Chapman (Eds.), *Coastal Marine ecology of temperate Australia* (pp. 152-171). Sydney: University of New South Wales Press.

Pearson, T. H. & Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Reveiw, 16*, 229-311.

Peterson, C. H. & Black, R. (1988). Density-dependant mortality caused by physical stress interacting with biotic history. *American Naturalist, 131*, 257-270.

Skilleter, G. A. (1995). Environmental disturbances. In A. J. Underwood & M. G. Chapman (Eds.), *Coastal marine ecology of temperate Australia* (pp. 263-276). Syndey: University of New South Wales Press.

Smith, B. (1995). *Tamar intertidal invertebrates*. Launceston: Queen Victoria Museum.

Snelgrove, P., Blackburn, T. H., Hutchings, P. A., Alongi, D. M., Grassle, J. F., Hummel, H., King, G., Koike, I., Lambshead, P. J. D., Ramsing, N. B. & Solisweiss, V. (1997). The importance of marine sediment biodiversity in ecosystem processes. *Ambio, 26*(8), 578-583.

Stark, J. S. (1998a). Heavy metal pollution and macrobenthic assemblages in soft sediments in two Sydney estuaries, Australia. *Marine Freshwater Research, 49*, 533-540.

Stark, J. S. (1998b). Effects of copper on macrobenthic assemblages in soft sediments: a laboratory experimental study. *Ecotoxicology, 7*, 161-173.

Underwood, A. J. (1989). The analysis of stress in natural populations. *Biological Journal Linnean Society, 37*, 51-78.

Ward, T. J. & Hutchings, P. A. (1996). Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Marine Ecology Progress Series, 135*, 123-135.

Wilson, J. G. & Jeffrey, D. W. (1994). Benthic biological pollution indices in estuaries. In J. M. Kramer (Ed.), *Biomonitoring of Coastal Waters and Estuaries.* (pp. 311-327). Boca Raton: CRC Press.

CHAPTER FOUR

Histological, growth and 7- ethoxyresorufin 0-deethylase (EROD) activity responses of greenback flounder *Rhombosolea tapirina* to contaminated marine sediment and diet

J.A. Mondon[1], S. Duda[2] and B.F.Nowak[1]

1 School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, Launceston, Tasmania 7250, Australia.

2 Key Centre for Applied and Nutritional Toxicology, Royal Melbourne Institute of Technology-University, Melbourne, Victoria 3000, Australia.

Accepted for publication in *Aquatic Toxicology*

ABSTRACT

Pathological abnormalities and mixed function oxygenase (MFO) enzymes changes are frequently used as indicators of anthropogenic contaminant exposure and effect. However, there is a paucity of research investigating the effects of contaminated sediment on native Australian benthic teleosts. As part of an ecotoxicological assessment of contaminated marine sediments in northern Tasmania, CYP1A induction, histological and growth response of greenback flounder, *Rhombosolea tapirina*, exposed to contaminated marine sediments were examined. Hatchery reared flounder were exposed to reference sediment, contaminated sediment or contaminated sediment and diet for 6 weeks. CYP1A induction, using the ethoxyresorufin-o-deethylase (EROD) assay, and histological and growth response in flounder were examined on cessation of the exposure trial. Significant differences were found between treatments in histological, growth and EROD response. Exposure to contaminated sediment and diet elicited multi-organ histological

response: principally partial and total epidermal erosion and multifocal necrosis of the liver. Prevalence of total epidermal erosion was greatest with exposure to disturbed contaminanted sediment (66.65% ± 16.65). Prevalence of multifocal necrosis of the liver was greatest with exposure to contaminanted sediment and diet (66.65% ± 16.65). Growth reduction, measured as percent growth inhibition, was evident in flounder exposed to contaminated sediment and diet (18.2% ± 11.99). Additionally, exposure to contaminated sediment and diet elicited elevated induction of the EROD liver detoxification enzyme (139.65 ± 24.22 pmol/min/mg protein) compared to exposure to contaminated sediment and non-contaminated diet (6.25 ± 0.81 pmol/min/mg) indicating presence and potential bioavailability of xenobiotics via food. Further, inhibited growth and histological alteration associated with exposure to contaminated sediment and diet suggest contaminants in Deceitful Cove sediment are cytotoxic.

Introduction

Benthic fish from urbanised and industrialised rivers and coastal regions are commonly exposed to long-term stress arising from exposure to sublethal contaminant concentrations. Bioindicators involving multiple levels of biological organisation can assess the effects of anthropogenic stressors in marine ecosystems. Long term exposure to sublethal concentrations can result in highly deleterious effects at both the individual and population level, due to subtle behavioural and morphological changes affecting feeding behaviours, reproductive success and ability to cope with stress (Murty, 1985). Industrial and urban pollution of the aquatic environment is known to adversely affect fish histology, predominantly targeting the liver, gills, spleen, kidney, reproductive organs and skin (for review see Hinton & Lauren, 1990). Exposure to specific organic contaminants via water (Peters *et al.*, 1996), diet and sediment (Courtney, 1980) results in CYP1A induction and formation of DNA-adducts implicated in chemically induced carcinogenesis (Stegeman & Lech, 1991).

Effects of short term contaminant exposure measured as the biochemical, physiological and histological response in benthic fish may provide a suite of biomarkers appropriate for longer term assessment and monitoring of sediment contamination. The commercially exploited greenback flounder (*Rhombosolea tapirina*) represents a suitable bioindicator species for assessment of Australian estuarine and shallow coastal marine waters. *R.tapirina* is commonly found in sheltered sandy habitats from 0-100m depth and exhibits a wide geographical distribution extending from southern Western Australia to southern New South Wales, Tasmania and New Zealand (Edgar, 1997). *R.tapirina* is a carnivorous, predatory, benthic species, which captures polychaetes and other prey by digging into the substrate (Edgar, 1997).

Deceitful Cove, in northern Tasmania is a popular recreational fishing area supporting a wide variety of pelagic and benthic fish species including the greenback flounder. A sediment quality triad (SQT) (Chapman, 1990) survey of Deceitful Cove, detected a link

between toxicity at the unicellular organism level and sediment contamination from the site (Mondon *et al.*, 1999a). As the main route of exposure for benthic organisms is generally via contact with contaminated sediment and diet, toxicity to benthic teleosts is highly likely. Extrapolation of toxicity from the unicellular to vertebrate level is difficult due to the paucity of work investigating the effects of contaminated sediment on native Australian fish species. Benthic species in immediate contact with sediment are thought to provide a more direct assessment of sediment contamination than pelagic species (Ankley *et al.*, 1991). Disturbance to contaminated sediment further complicates environmental assessment and management decisions for this particular site: the impact of proposed dredging with respect to potentially increasing toxicity of sediment contaminants is not clear. However, the results from a recent assessment of immune response of greenback flounder to contaminated sediment and diet indicates potential immunotoxicity of sediment from Deceitful Cove (Mondon *et al.*, 1999b). Further, there is strong evidence to suggest immunotoxicity is enhanced by sediment disturbance.

Ecotoxicological field data on this species is not available. The effects of contaminated sediment, diet and sediment disturbance on EROD induction, histology and growth of greenback flounder were assessed in order to determine the main route of toxicity of contaminants, the bioavailability of xenobiotic contaminants, and to provide background data for future assessment of the sediment remediation.

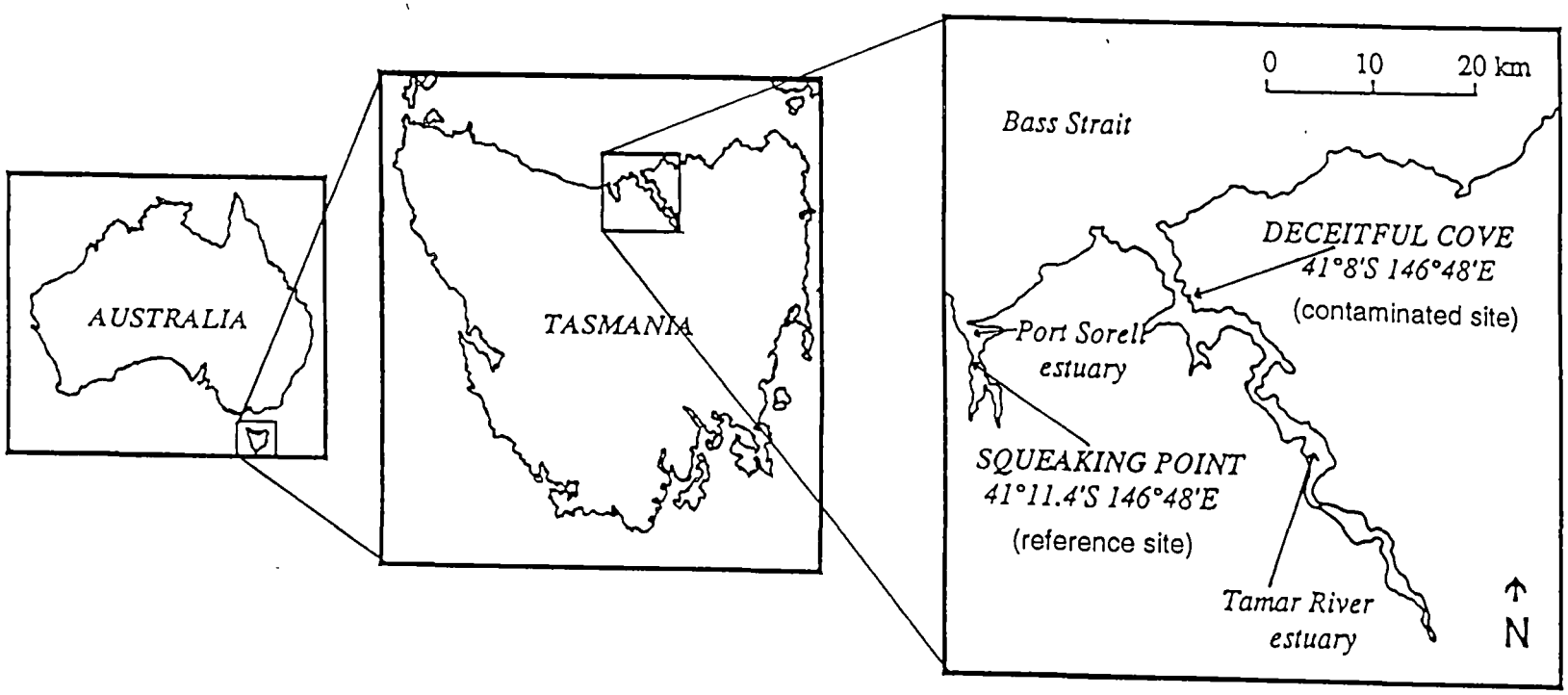


Figure 1. Location area of contaminated sediment and reference sampling sites in northern Tasmania.

Materials and methods

Hatchery reared flounder were exposed to shallow sub-tidal sediment from contaminated and non-contaminated estuarine sites. Sediment disturbance was simulated under laboratory conditions, and the impact of exposure to diet and sediment from the contaminated site evaluated.

Sexually immature, twelve month old greenback flounder (44.31±2.9g (mean ± S.E.)) individuals were acclimated to 17(±2) ppt salinity for two weeks at 15°C under 12:12 hour light dark regime and fed with Pivot (previously known as Gibson's) flounder pellets (Pivot Limited, Australia). Post-acclimation the fish were weighed, then divided into 5 experimental treatment groups (Table 1).

Table 1. Experimental treatment groups.

Treatment	Sediment	Diet	Dredging simulation	Mesh size and position	Treatment referral in text
1	reference	non-contaminated	no	0.5 mm, in contact with sediment	reference sediment/normal diet
2	contaminated	non-contaminated	no	0.25 mm, suspended above sediment - not in direct contact	screened sediment/normal diet
3	contaminated	non-contaminated	yes	0.5 mm, in contact with sediment	disturbed contaminated sediment/normal diet
4	contaminated	non-contaminated	no	0.5 mm, in contact with sediment	contaminated sediment/normal diet
5	contaminated	contaminated	no	0.5 mm, in contact with sediment	contaminated sediment/ contaminated diet

Replicated experimental treatments comprised of two tanks with 10 fish per 250 L tank, flow through aerated 16.5(±0.5) ppt filtered seawater, plus eight litres of sediment spread at approximately 10 cm depth (Figure 2). Contaminated (Deceitful Cove) and reference (Port Sorell) sediments (Figure 1) were selected based on geochemical data collected during the SQT survey (Table 2). A 5 mm mesh was placed on top of the sediment in treatments 1, 2, 4 and 5 to reduce resuspension of sediment whilst allowing direct contact between sediment and fish during feeding and rest. A suspended 0.25 mm mesh was used to inhibit direct contact with sediment for experimental treatment 2. Simulated dredging activity in treatment 3 involved moving the mesh to one side of the tank, scooping up sediment from the bottom of the tank by hand, and releasing it at the surface, twice daily, for approximately 10 minutes duration. Water quality parameters were monitored daily: dissolved oxygen (7.64 ± 0.05 mg/L), ammonia (0.25 ± 0.001 ppm), temperature (15.64 ± 0.05 °C) and salinity (16.39 ± 0.18 ppt).

All fish were fed approximately 4.2 g feed/fish/day (dry weight), or approximately 9% of body weight per day. Feed comprised a homogenised mixture of 1:30:30 gelatine, flounder pellets and farmed Pacific oyster (*Crassostrea gigas*) meat (normal diet), or meat of wild Pacific oysters collected from Deceitful Cove (contaminated diet). Uneaten food was removed from tanks at the end of each day (mass not determined). After a 6 week exposure the fish were euthanised without recovery (benzocaine 150 ppm) weighed and samples for histology, EROD and chemical analysis were collected.

Table 2 **Mean (± S.E.) levels of trace metals, polycyclic aromatic hydrocarbons and organochlorines found in oysters (*Cassostrea gigas*) and sediment from Deceitful Cove and Squeaking Point: 1996-1998 (Mondon *et al.*, 1999a ; Mondon *et al.*, 1999c).**

Contaminant	Deceitful Cove Sediment	Deceitful Cove Oyster	Squeaking Point Sediment
Trace metals	($mg\ kg^{-1}$) dry weight		($mg\ kg^{-1}$) dry weight
Ag	0.178 ± 0.04	NA	0.04 ± 0.01
Al	12660.8 ± 2643	NA	3217.7 ± 156.7
Cd	1.93 ± 0.49	NA	0.06 ± 0.01
Cr	14.6 ± 1.14	NA	10.44 ± 0.32
Cu	26.83 ± 3.1	NA	3.79 ± 1.25
Fe	11195.7 ± 840.2	NA	6485.2 ± 272.5
Mn	57215.9 ± 10808	NA	45.1 ± 2
Ni	75.09 ± 20.86	NA	3.07 ± 0.24
Pb	322.28 ± 68.5	NA	8.39 ± 1.74
Zn	1064.18 ± 235.16	NA	10.39 ± 0.98
Polycyclic aromatic hydrocarbons	($\mu g\ kg^{-1}$) dry weight		
Σ PAHs	6534.37 ± 1616.9	NA	NA
Organochlorines	($ng\ g^{-1}$) dry weight	($ng\ g^{-1}$) fat weight	($ng\ g^{-1}$) dry weight
Σ PCBs	1173.75 ± 508.3	2762.1 ± 1179.1	0
Σ DDT	8.45 ± 2.3	68.02 ± 30	0.8 ± 0.8

NA - not available.

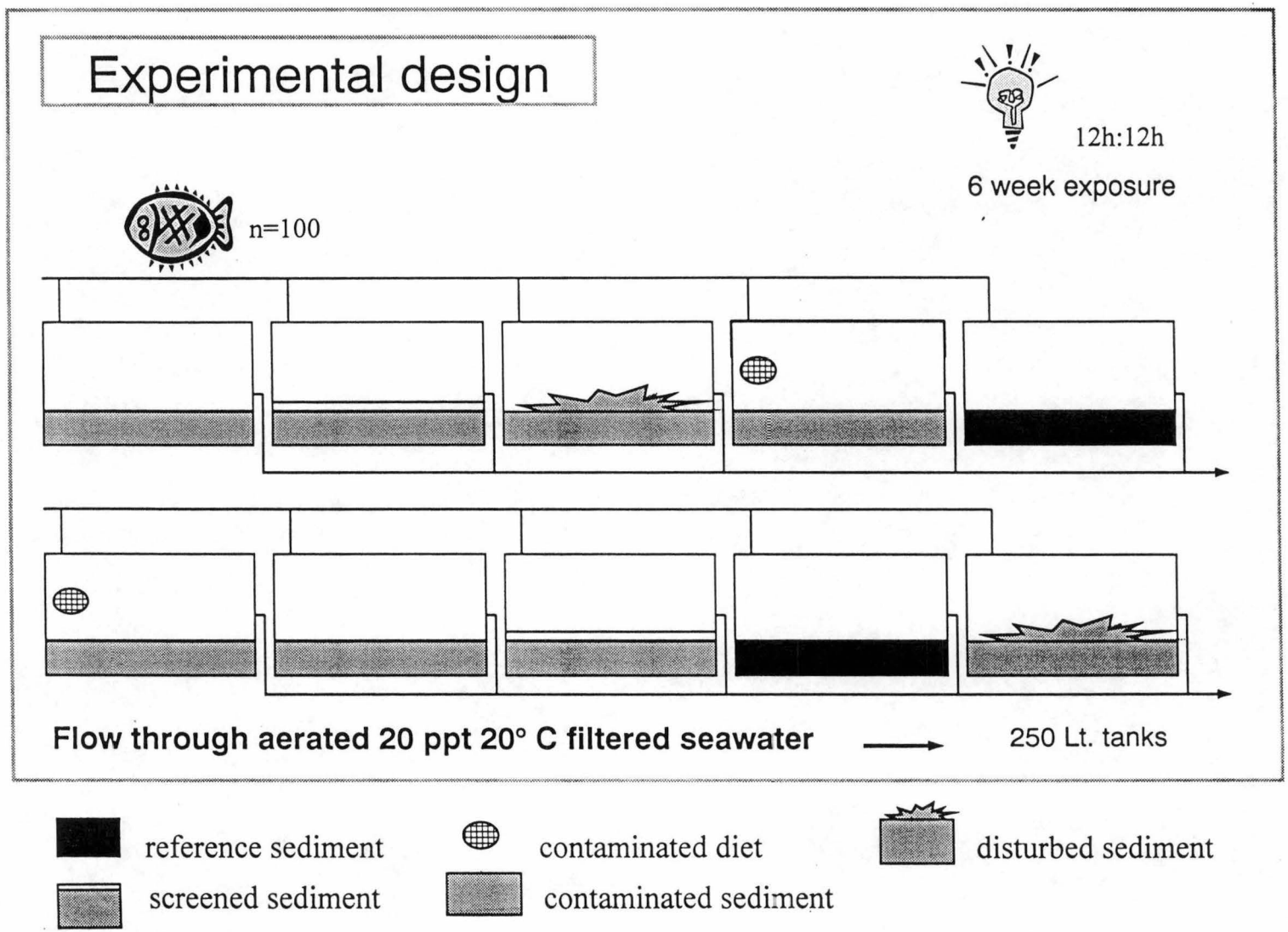


Figure 2. Experimental design of greenback flounder exposure to contaminated sediment and diet. (Not submitted for publication).

Histology

The first gill arch, kidney, spleen, liver, heart, and skin with muscle tissue from the underside of the mid region of the flounder were excised and fixed in 10% buffered formalin for histological analysis. Once fixed, the tissues were dehydrated through a graded series of ethanol solutions up to 100%. Tissue was cleared in xylene prior to embedding in paraffin. Tissue was sectioned using a MICROM Heidelberg microtome at 4μm. Haematoxylin and eosin (H&E) stained sections and periodic acid Schiff (PAS) stained sections were prepared from each tissue block. Sections were examined "blind" at x100 and x400 magnification using an Olympus BH-2 stereomicroscope. Histological abnormalities detected in the gill, liver, heart, skin, spleen and kidney were recorded as presence or absence per fish and expressed as percentage of fish affected (prevalence) per tank. Changes in gill lamellae of each fish were quantified along 2 complete gill filament sections and recorded as present or absent. Mucous and chloride cell proliferation was determined as number of cells per 10 lamella units in PAS stained sections (Powell *et al.*, 1995; Powell & Perry, 1997). Histological alterations in H&E stained liver, heart, skin and kidney whole sections were recorded as present or absent. Melanomacrophage frequency and area (number and area of aggregates per mm^2) in spleen was assessed from PAS stained sections using image analysis (Analytical Imaging Station). Only one section from each block was examined to ensure independence of data.

7-Ethoxyresorufin O-deethylase (EROD) assay (S9 fraction)

Liver samples were excised from each fish, placed in cryogenic vials, frozen immediately in liquid nitrogen and stored at -80°C prior to analysis. All steps in S9 fraction preparation were performed at 4°C. Livers were thawed, weighed individually and then homogenised in 0.1M phosphate buffer, pH 7.4, containing 1 mM dithiothreitol, 1 mM ethylenediamine tetraacetic acid (EDTA), 0.1 mM phenanthroline, and 0.1 mM KCl, 20% w/v. Homogenates were centrifuged at 10,000 rpm for 20 minutes. The supernatant (S9 fraction) was retained and stored overnight at -80°C.

EROD activity of the S9 fraction was evaluated flourometrically (Hitachi F4500 Flourescence spectrometer) using the method of Burke and Mayer (1974), modified as described by Holdway *et al.* (1994). Protein assays were performed on the supernatant using the method of Lowry *et al.* (1951).

Chemical analysis

Liver and gill tissues were excised for chemical analysis. For trace metals analysis, liver and gill tissue samples from individual fish were dried overnight at 70°C, then digested in nitric acid at 100°C for 4 hours and analysed by ICP-Optical Emission Spectrometer (ICP-OES), detection limit 0.01 mg kg^{-1} dry weight. Gill samples were pooled due to small mass. For PAH and PCBs analysis, liver and gill tissue was wrapped in aluminium foil and frozen to -20°C. Lipids were extracted from both sets of samples by homogenisation with 1:1 acetone/hexane and cleaned with concentrated sulphuric acid. PCB compounds were separated and quantified by gas chromatography (Varian with ECD-detector), detection limit 10 mg kg^{-1} dry weight. Internal standard tetrachlorobenzene was used in conjunction with Arochlor 1254 as an external standard. Diets were dried overnight at 70°C, ground, digested in nitric acid and analysed by ICP-OES for trace metals, detection limit 0.1 mg kg^{-1} dry weight. For PAH detection diet samples were mixed with sodium sulphate and crushed, dichloromethane/acetone was then added, the samples sonicated and shaken, and the extract analysed by gas chromatography - mass spectrum (GC-MS), detection limit 0.2 mg kg^{-1} dry weight.

Statistical analysis

Growth response and histology alterations quantified in gills, heart, kidney, spleen, skin and livers of flounder were analysed by analysis of variance (ANOVA) using the statistical package JMP (SAS Institute Inc.,1995). The nested experimental design assessed effects of treatments and tanks (nested within treatments). Shapiro-Wilk W and Cochran's test were used to test for normality and homogeneity of variances respectively. Where data were heterogeneous log (x+1) transformation was used. Where data were intractably

heterogenous after transformation, the data were tested untransformed but interpreted with caution (Underwood, 1981). If significant differences were detected, the Tukey-Kramer comparison of means test was used.

Results

Histology and growth

Exposure to contaminated sediment and diet resulted in histological alterations in flounder. The prevalence of histological responses increased markedly with exposure to disturbed contaminated sediment and diet, and decreased marginally when not in direct physical contact with contaminated sediment (Table 3).

The greatest prevalence of histological response to contaminated sediment involved multifocal hepatocellular coagulation necrosis and inflammation of the liver (Figure 3). Whilst multifocal necrosis was present in all treatments, exposure to disturbed contaminated sediment/normal diet elicited the highest significant prevalence of liver necrosis, whereas prevalence of inflammation peaked in flounder exposed to contaminated sediment/contaminated diet (Table 3). Necrotic cardiac tissue was evident in all non-reference experimental treatments. However, the prevalence of necrosis of the myocardium tissue increased with exposure to contaminated sediment/contaminated diet. Prevalence of total epidermal erosion (Figure 4) was significantly higher in disturbed contaminated sediment/normal diet and contaminated sediment/contaminated diet treatments. Disturbed sediment/normal diet elicited the greatest prevalence of epidermal loss with approximately 67% of the population exhibiting total loss compared to 33% exposed to contaminated sediment/contaminated diet. A trend towards increased partial epidermal loss (Figure 4) when exposed to contaminated sediment/contaminated diet was also evident. Similarly, a trend towards increased prevalence of renal necrosis and tubular vacuolation associated with contaminated sediment/contaminated diet was present. Renal tubule vacuolation was prevalent in all non-reference experimental treatments.

Lamellar epithelial hyperplasia was present in gills from experimental treatments, with the highest prevalence occurring in flounder exposed to contaminated sediment/normal diet (Figure 5). Lamellar fusion occurred in flounder exposed to disturbed contaminated sediment/normal diet, screened contaminated sediment/normal diet and contaminated sediment/normal diet only (Figures 5 & 6).

Table 3. **Prevalence of histological response to contaminated sediment and diet exposure in greenback flounder *Rhombosolea tapirina*. Data presented as percentages, means from 2 replicate tanks / treatment (6 fish / tank) (± S.E.). * denotes percentage of fish exhibiting histological response significantly greater than reference (ANOVA, $p<0.05$).**

Organ	Pathology	1 Reference sediment/ non-contaminated diet	2 Screened contaminated sediment/non-contaminated diet	3 Disturbed contaminated sediment/non-contaminated diet	4 Contaminated sediment/non-contaminated diet	5 Contaminated sediment/ contaminated diet
Gills	telangiectasis	8.3 ± 8.3	16.6 ± 0	16.6 ± 0	8.3 ± 8.3	8.3 ± 8.3
	lamellar fusion	0	16.6 ± 0	8.3 ± 8.3	16.6 ± 16.6	0
	epithelial hyperplasia	0	8.3 ± 8.3	16.6 ± 0	33.3 ± 0*	8.3 ± 8.3
	bifurcated lamella	0	0	0	8.3 ± 8.3	0
Liver	multifocal necrosis	8.3 ± 8.3	33.3 ± 16.7	41.6 ± 8.4	50 ± 0*	33.3 ± 0
	inflamation	0	16.6 ± 0	16.6 ± 0	16.6 ± 0	33.3 ± 16.7*
Heart	necrosis	0	16.6 ± 0	24.95 ± 8.35	24.95 ± 8.35	33.3 ± 16.7
	fibrosis	0	8.3 ± 8.3	8.3 ± 8.3	0	0
Skin	epidermal loss (partial)	0	0	8.3 ± 8.3	16.6 ± 0	24.95 ± 8.35
	epidermal loss (total)	0	0	66.65 ± 16.65*	0	33.3 ± 0*
Spleen	edema	0	0	24.95 ± 8.35*	0	0
	necrosis	0	0	8.3 ± 8.3	0	0
	cyst	0	0	0	8.3 ± 8.3	0
Kidney	tubule vacuolation	0	24.95 ± 8.35	24.95 ± 8.35	24.95 ± 8.35	33.3 ± 0
	necrosis	8.3 ± 8.3	16.6 ± 0	0	8.3 ± 8.3	24.95 ± 8.35
	granuloma	0	8.3 ± 8.3	8.3 ± 8.3	8.3 ± 8.3	0

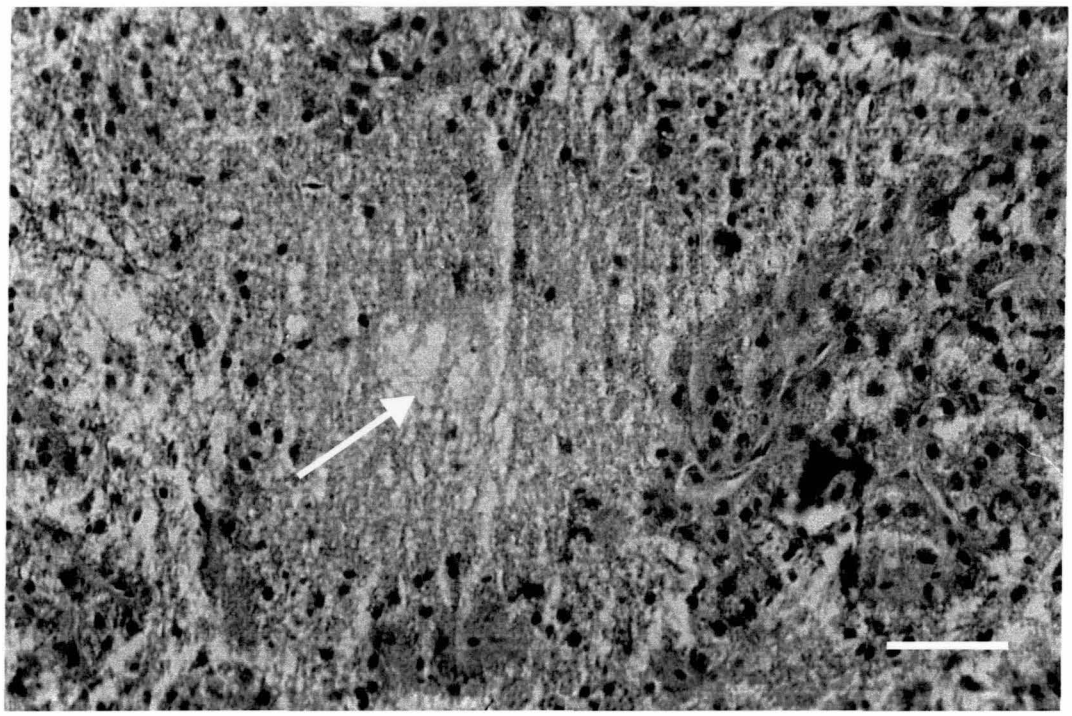
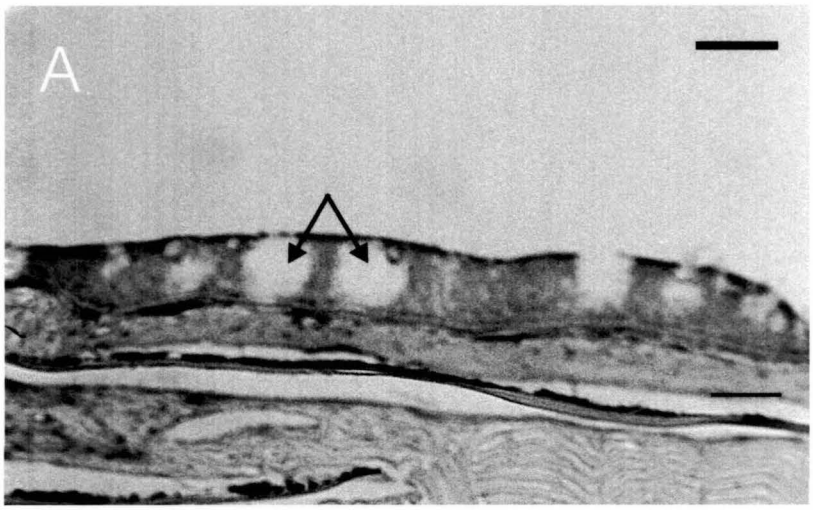
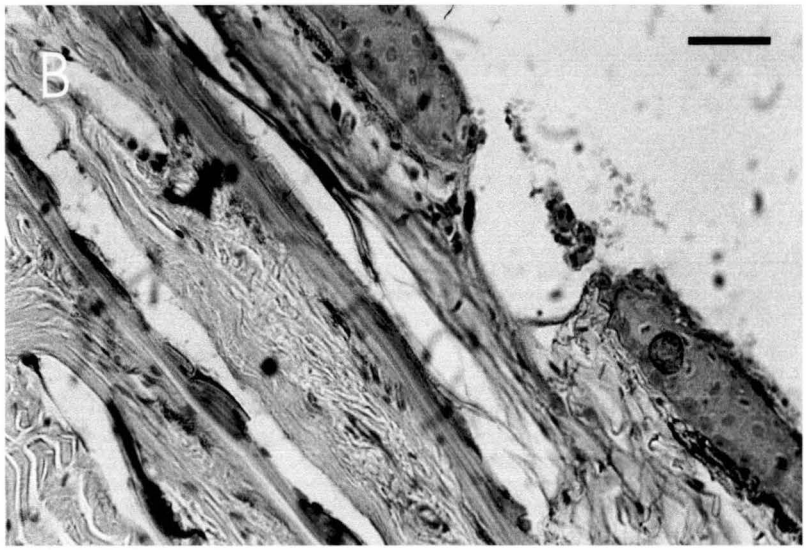


Figure 3. **Liver of greenback flounder *Rhombosolea tapirina* exhibiting coagulative necrosis following 6 weeks of constant exposure to contaminated sediment/contaminated diet. H&E stain. Arrow indicates the centre of the necrotic region. Bar represents 26 μm.**

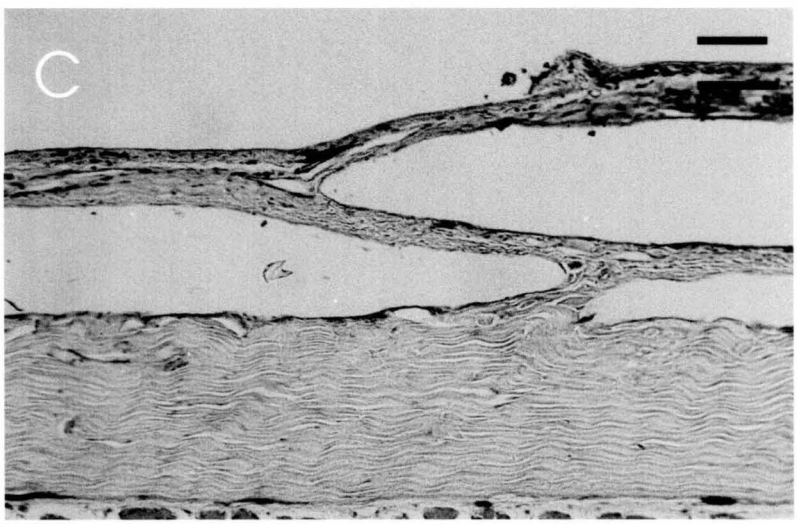
Figure 4. Ventral skin of greenback flounder *Rhombosolea tapirina* exhibiting:



A) Partial epidermal loss following exposure to contaminated sediment/non-contaminated diet. Arrows indicate necrotic region of epidermis. H&E stain. Bar represents 72 μm.



B) Partial epidermal loss following exposure to contaminated sediment/non-contaminated diet. H&E stain. Bar represents 18 μm.



C) Total epidermal loss following exposure todisturbed contaminated sediment/non-contaminated diet. H&E stain. Bar represents 72 μm.

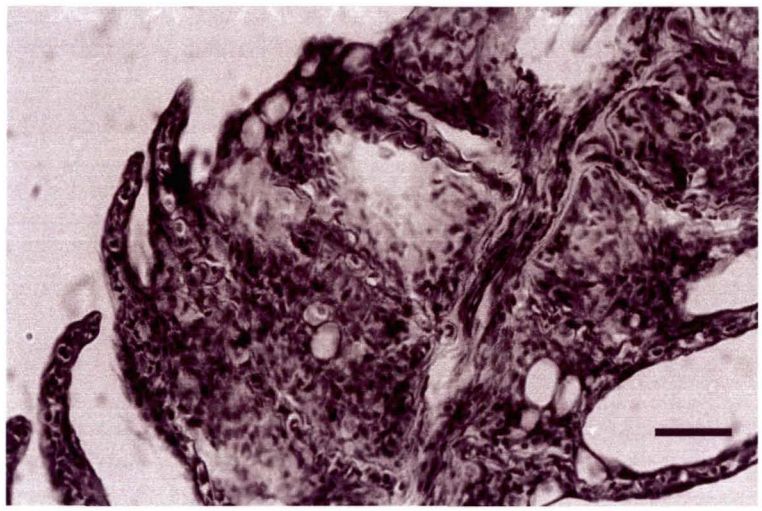


Figure 5. **Gill lamellae of greenback flounder *Rhombosolea tapirina* exhibiting fusion and epithelial hyperplasia following exposure to disturbed contaminated sediment/non-contaminated diet. H&E stain. Bar represents 26 μm.**

Figure 6. **Gill lamellae of greenback flounder *Rhombosolea tapirina* exhibiting fusion and epithelial hyperplasia following exposure to disturbed contaminated sediment/non-contaminated diet. H&E stain. Bar represents 26 μm.**

Chloride cell proliferation was significantly higher in disturbed contaminated sediment/normal diet and contaminated sediment/contaminated diet treatments (Table 4). Treatment did not significantly affect mucous cell number (Table 4). However, a trend towards increased number of mucous cells in disturbed contaminated sediment/normal diet was evident (Table 4; Figure 6).

Vacuolation (edema) of the spleen only occurred in flounder exposed to disturbed contaminated sediment/normal diet. Melanomacrophage centre number and area in spleen were significantly higher in flounder exposed to contaminated sediment in all treatments (Table 5).

Table 4. Mean (± S.E.) number of cells / lamellar unit for greenback flounder *Rhombosolea tapirina* exposed to contaminated sediment and diet. * denotes value significantly greater than reference (ANOVA, p<0.05)

Treatment	Cell type	
	Mucous	Chloride
1 Reference sediment/non-contaminated diet	22.63 ± 1.67	14.68 ± 0.58
2 Screened contaminated sediment/non-contaminated diet	24.94 ± 2.05	15.87 ± 0.97
3 Disturbed contaminated sediment/non-contaminated diet	30.41 ± 2.97	19.76 ± 0.97*
4 Contaminated sediment/non-contaminated diet	19.3 ± 1.01	14.78 ± 0.53
5 Contaminated sediment/ contaminated diet	20.17 ± 2.06	20.24 ± 1.2*

Table 5. Mean (± S.E.) melanomacrophage aggregate response in spleen of greenback flounder *Rhombosolea tapirina* exposed to contaminated sediment and diet. Different letters denote statistically different values (ANOVA, p<0.05).

Response	1 Reference sediment/non-contaminated diet	2 Screened contaminated sediment/non-contaminated diet	3 Disturbed contaminated sediment/non-contaminated diet	4 Contaminated sediment/non-contaminated diet	5 Contaminated sediment/ contaminated diet
% area	1.47 ± 0.41 ^{a}	7.03 ± 1.44 ^{b}	5.19 ± 1.27 ^{b}	6.12 ± 1.92 ^{b}	6.08 ± 1.33 ^{b}
No./mm^2	3.75 ± 0.78 ^{a}	6.58 ± 0.62 ^{b}	6.1 ± 0.43 ^{b}	7.0 ± 0.75 ^{b}	7.54 ± 0.76 ^{b}

The average growth (weight change/tank(± S.E.)) of flounder significantly decreased in fish exposed to contaminated sediment/contaminated diet (Figure 7). Additionally, a trend towards weight loss in fish exposed to disturbed contaminated sediment/normal diet was evident. The feeding behaviour of flounder was monitored daily, with little evidence of the contaminated diet being unpalatable. Both diets (contaminated and non-contaminated) were rapidly engulfed without regurgitation.

7-Ethoxyresorufin-O-deethylase (EROD) assay

Screened contaminated sediment/normal diet and contaminated sediment/contaminated diet treatments elicited significantly higher EROD induction compared to contaminated sediment/normal diet and reference sediment/normal diet treatments (Figure 8). Exposure to screened sediment/normal diet resulted in an eight to twelve fold increase in activity; contaminated sediment/contaminated diet elicited a ten to sixteen fold increase. There was no significant difference in EROD activity between reference sediment/normal diet, disturbed contaminated sediment/normal diet and contaminated sediment/normal diet exposure. Similarly, differences between disturbed and undisturbed contaminated sediment were non-significant. However, despite variation between replicates, a trend exhibiting a three to four fold increase in EROD activity with exposure to disturbed sediment compared to non-disturbed sediment was present.

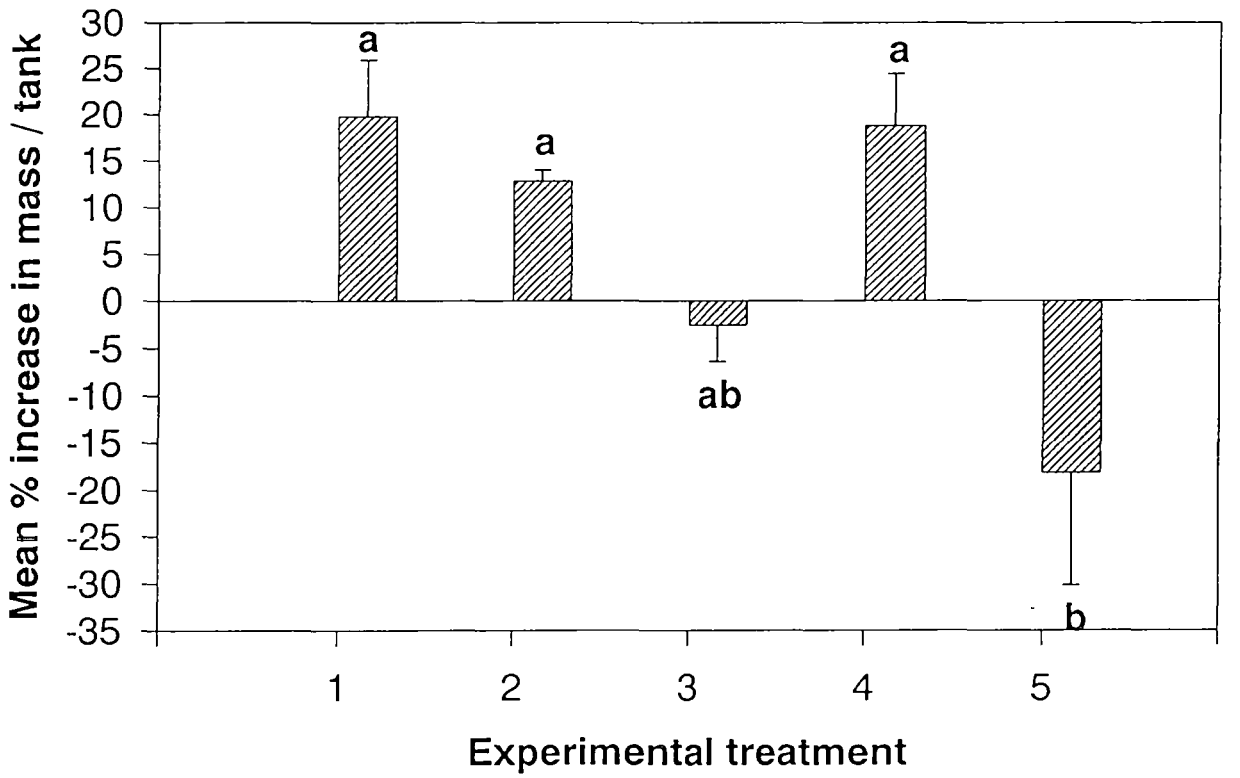


Figure 7. **Growth response to contaminated sediment and diet exposure in greenback flounder *Rhombosolea tapirina*: 1. reference sediment/non-contaminated diet, 2. screened contaminated sediment/non-contaminated diet, 3. disturbed contaminated sediment/non-contaminated diet, 4. contaminated sediment/non-contaminated diet, 5. contaminated sediment/contaminated diet. Values are expressed as mean (± S.E.); n=2 replicates /treatment. Different letters indicate statistically significant differences ($p<0.05$).**

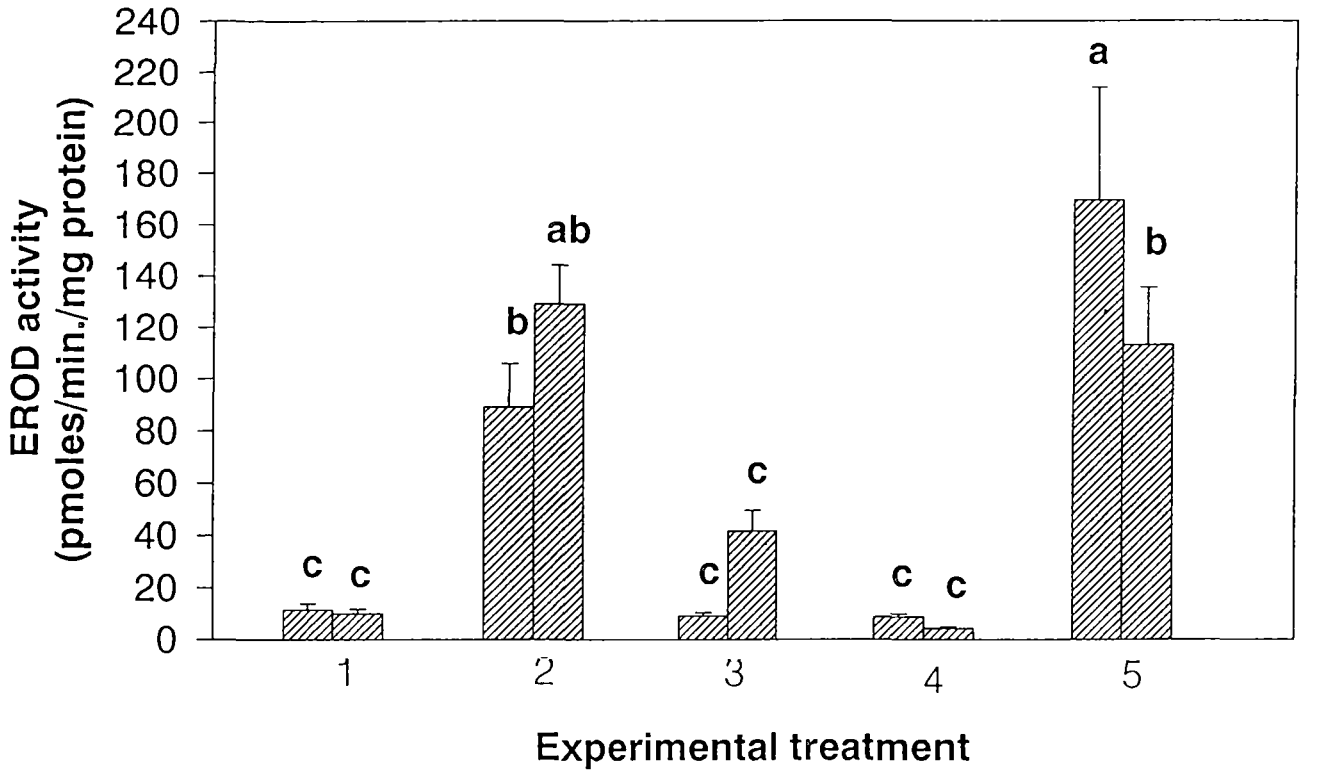


Figure 8. EROD response to contaminated sediment and diet exposure in greenback flounder *Rhombosolea tapirina*: 1. reference sediment /non-contaminated diet, 2. screened contaminated sediment/non-contaminated diet, 3. disturbed contaminated sediment/non-contaminated diet, 4. contaminated sediment/non-contaminated diet, 5. contaminated sediment/contaminated diet. Values are expressed as mean (± S.E.); n=20 replicates/treatment. Different letters indicate statistically significant differences ($p<0.05$).

Chemical analysis

Trace metals in the contaminated diet were detected at levels ranging from 1.3 to 10.9 times greater than trace metal levels in the non-contaminated diet (Table 6). Copper exhibited the greatest increase in contaminated versus non-contaminated diet, followed by nickel, zinc and aluminium. Fluoranthene, pyrene, benzo(*a*)anthracene, chrysene and benzo(*a*)pyrene were detected only in the contaminated diet (Table 6).

Table 6. Trace metals and PAH levels detected in contaminated and non-contaminated flounder diets.

Contaminant	Detection limit	Contaminated diet	Non-contaminated diet
Trace metals (mg kg^{-1} dry wt.)	(ppm)		
Al	0.1	107.47	35.46
As	0.1	3.77	2.46
Cd	0.1	3.98	1.96
Cr	0.1	1.41	0.37
Cu	0.1	245.06	22.375
Fe	0.1	336.86	262.56
Mn	0.1	105.45	80.52
Ni	0.1	0.86	0.18
Pb	0.1	1.7	<0. 1
Sn	0.1	8.45	6.62
Zn	0.1	1114	248.65
PAHs (mg kg^{-1} dry wt.)			
Acenaphthylene	0.2	nd	nd
Anthracene	0.2	nd	nd
Benzo(*a*)anthracene	0.2	0.2	nd
Benzo(*b*)fluoranthene	0.2	nd	nd
Benzo(*k*)fluoranthene	0.2	nd	nd
Benzo(*a*)pyrene	0.2	0.3	nd
Benzo(*ghi*)perylene	0.2	nd	nd
Chrysene	0.2	0.2	nd
Dibenzo(*ah*)anthracene	0.2	nd	nd
Fluoranthene	0.2	0.4	nd
Fluorene	0.2	nd	nd
Indeno(*123-cd*)pyrene	0.2	nd	nd
Naphthalene	0.2	nd	nd
Phenanthrene	0.2	nd	nd
Pyrene	0.2	0.6	nd

nd - not detected

Flounder exposed to contaminated diet exhibited significantly elevated levels of As in the liver ($p<0.001$), whereas other trace metal concentrations in gill and liver tissue exhibited non-significant differences due to high variation within treatments (Table 7). However, several trends are apparent. Elevated levels of Mn, Cr, Cu, Fe, Pb and Zn were evident in the liver of flounder exposed to contaminated diet, whereas gill tissue from the same treatment displayed elevated Fe, Mn, Sn and Zn. Fish exposed to disturbed sediment exhibited a trend towards elevated Al, Fe and Cu in the gills, and Cr, Fe, Ni, Sn and Zn in the liver. PCB and PAH concentrations in liver and gill tissue were below detection limits.

Table 7. Mean (± S.E.) trace metals concentrations in gill and liver for greenback flounder *Rhombosolea tapirina*, (mg kg^{-1} dry wt.). * indicates statistically significant difference (ANOVA, $p<0.05$)

Tissue	Metal	1 Reference sediment/non-contaminated diet	2 Screened contaminated sediment/non-contaminated diet	3 Disturbed contaminated sediment/non-contaminated diet	4 Contaminated sediment/non-contaminated diet	5 Contaminated sediment/ contaminated diet
Gill	Al	0	0	167.79 ± 125.32	26.14 ± 26.14	3.14 ± 3.14
	Cd	0	0.74 ± 0.74	0	0	0.37 ± 0.37
	Cr	0	7.53 ± 7.09	0	9.89 ± 9.89	0
	Cu	5.88 ± 3.47	7.29 ± 7.29	10.71 ± 6.65	6.31 ± 5.27	7.85 ± 4.6
	Fe	99.82 ± 57.9	116.7 ± 67.65	164.97 ± 96.95	127.3 ± 75.33	196.12 ± 125.31
	Mn	0	0	7.99 ± 7.99	3.26 ± 3.19	77.09 ± 77.05
	Ni	0.11 ± 0.11	0	0	3.75 ± 3.75	2.85 ± 2.85
	Pb	1.01 ± 1.0	2.15 ± 1.98	0	8.87 ± 7.6	0
	Sn	0	0	0.013 ± 0.01	0	312.39 ± 312.39
	Zn	54.37 ± 31.53	72.19 ± 44.52	53.94 ± 32.07	64.54 ± 47.66	185.83 ± 108.2
Liver	Al	2.04 ± 0.97	7.15 ± 2.77	5.76 ± 3.5	5.64 ± 3.54	6.40 ± 6.39
	Cd	0.09 ± 0.09	0.92 ± 0.39	0	0.28 ± 0.24	0.55 ± 0.31
	Cr	0.14 ± 0.14	0.35 ± 0.13	1.25 ± 0.45	0.33 ± 0.23	1.23 ±0.86
	Cu	66.39 ± 6.3	58.78 ± 9.44	73.47 ± 11.76	54.82 ± 9.088	98.01 ± 20.37
	Fe	33.62 ± 5.34	28.98 ± 3.17	38.57 ± 6.65	30.85 ± 5.69	56.31 ± 10.24
	Mn	4.07 ± 2.08	5.46 ± 1.273	5.56 ± 1.35	5.03 ± 2.6	31.86 ± 22.34
	Ni	1.86 ± 0.87	7.56 ± 3.11	12.33 ± 0.18	0.58 ± 0.58	0.71 ± 0.71
	Pb	14.94 ± 8.6	2.68 ± 0.88	2.27 ± 1.44	11.90 ± 9.76	22.52 ± 13.77
	Sn	0	0	309.33 ± 305.67	0	0
	Zn	74.81 ± 7.86	67.84 ± 6.34	102.51 ± 15.84	72.54 ± 10.69	103.54 ± 14.71
	As	1.9 ± 0.09	2.76 ± 0.42	0.46 ± 0.15	2.55 ± 0.02	9.21 ± 1.66*

Discussion

Greenback flounder exposed to contaminated sediment and diet exhibited a suite of histological, growth and EROD responses. The liver displayed the highest prevalence of histological change, with necrosis representing the dominant structural alteration. Hepatic necrosis and inflammation indicative of infection or toxic injury by contaminants (Hinton & Lauren, 1990) was prevalent in flounder exposed to contaminated sediment and diet. The prevalence of necrosis suggests that poor liver condition in exposed flounder is related to complex contaminant exposure from Deceitful Cove sediment. Granulomas of unknown aetiology were present in the kidney of three fish indicating a chronic inflammatory response. However, given the low prevalence, granuloma occurrence may not be treatment related.

The higher prevalence of chloride cell hyperplasia, epithelial hyperplasia and lamellar fusion in exposed flounder parallels findings in freshwater species exposed to similar contaminants in the field. Buffalo fish (*Ictiobus bubalus* and *Ictiobus cyprinellus*) collected from a river basin contaminated with multiple metals and organics, exhibited chloride, mucous and epithelial cell hyperplasia and lamellar fusion (Thiyagarajah *et al.*, 1996). Similar structural changes were found in trout (*Salmo trutta*) exposed to heavily polluted stream water (Pawert *et al.*, 1998). As gills provide the most extensive interface with the aquatic environment (Heath, 1987) it is not unexpected that structural changes were present. Although sediment disturbance and corresponding turbidity did not increase the number of mucous cells relative to non-disturbed and screened sediment, chloride cell numbers were significantly higher. Several metals have been implicated in chloride cell proliferation, principally Cd, Cu and Zn (Baker, 1969; Matthiessen & Brafield, 1973; Oronsaye & Braefield, 1984). The greater prevalence of chloride cell proliferation may well be due to elevated exposure to these contaminants via disturbance and diet. However, non-stimulation of mucous secretion indicates the levels of these contaminants are most likely below acute concentrations (Heath, 1987). The gross appearance of gills was that of healthy fish and the observed microscopic changes were moderate which indicates gill function was most likely not seriously affected.

The increases in melanomacrophage frequency and size noted in the experimental treatments suggests a strong link between exposure to contaminated sediment and an increase in size and number of melanomacrophage centres. In other field and mesocosm studies, flounder (*Platichthys flesus*) exposed to contaminated dredge spoil and sediments, and grenadier (*Coryphaenoides rupestris*) exposed to deep sea sediments exhibited a positive trend between a marked increase in size of melanomacrophage aggregates and an increase in sediment contamination levels (Lindesjoo *et al.*, 1996; Vethaak *et al.*, 1996a; Vethaak and Wester, 1996b). Exposures to sediments contaminated with polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyl (PCBs) are known to elicit melano-macrophage proliferation in carp (*Cyprinus carpio*) (van der Weiden *et al.*, 1993). In the absence of supporting chemical information for this study, melanomacrophage proliferation is viewed as a non-specific indicator of stress related to, directly or indirectly, sediment contaminant exposure.

Considering the degree of growth impairment of flounder exposed to contaminated diet in this study, it is highly probable that the survival potential of the flounder has been severely compromised (Depledge, 1993). If pollution-induced impairment exists in the field, it is likely to be of direct relevance to the survivorship of the wild population (Depledge *et al.*, 1995). The recorded decrease in growth is consistent with exposure to a metal contaminated diet. Trophic transfer studies investigating the effect of diets high in metals have found that trout (*Salmo trutta*) on metals-contaminated diets exhibit reduced growth, inhibited feeding activity and induced constipation (Pascoe *et al.*, 1994;Woodward *et al.*, 1995). Despite the greenback flounder being fed to satiation, it is unknown whether the fish exposed to the contaminated diet actually consumed less than those exposed to the non-contaminated diet. Nonetheless, equal amounts of food were available for consumption in all treatments as controls.

Reduced growth and nutrient deficiency has also been linked to epidermal atrophy / exfoliation (Taveekijakarn *et al.*, 1996). Partial and total epidermal loss exhibited in

flounder exposed to contaminated diet may in part be linked to a nutritional deficiency associated with high metal consumption. In the absence of a diet elevated in metals, atrophy prevalence in disturbed sediment conditions indicates a stress-related pathology possibly associated with loss of appetite and dermal abrasion. However, direct exposure to non-disturbed contaminated sediment can also result in some degree of atrophy of the epidermal layer and points to unspecified sediment associated factors, which may involve metals eliciting epidermis loss. Mechanisms of metal excretion are also thought to involve mucous secretion (Heath, 1987). Inability to utilise a major excretion route (epidermal mucus excretion) would in the short term inhibit the removal rate of hazardous chemicals by the fish, thereby raising body burdens of contaminants. Apart from precipitation of toxic ions, maintenance of a complete epidermal mucous secreting layer is also vital for ion and water regulation, physical protection, boundary surface layer modification when swimming, and disease protection (Shephard, 1994).

Elevated EROD activity, induced in fish by organics such as polycyclic aromatic hydrocarbons (PAHs), PCBs, PCDDs and PCDFs (Hahn *et al.*, 1992; van der Weiden *et al.*, 1993), implies exposure and uptake by flounder of at least one or more xenobiotic contaminants. Despite non-detection of organics in gill and liver tissue samples, most probably due to the high detection levels in the present study (10 mg/kg dry wt.), induction of the EROD liver detoxification enzyme response in contaminated diet treatment indicates bioavailability of xenobiotics from Deceitful Cove oyster meat. Inference associated with elevated EROD activity in screened sediment treatment is less clear. The data suggest potential bioavailability and toxicity of contaminants released from sediments to overlying water is greater than direct exposure to contaminated sediment: implying contaminant uptake via the sediment is negligible. If correct, EROD data from screened versus non-screened contaminated sediment repudiates histological data where an increased prevalence of cellular and tissue alteration of the liver in particular, was associated with direct exposure to contaminated sediment. Furthermore, the finding is contrary to similar benthic teleost studies where organic contaminant uptake via direct ingestion of sediment is identified as a major route of exposure (Courtney, 1980). Fish feeding in contaminated

sediments exhibited contaminant concentration levels five times higher than fish consuming contaminated prey in uncontaminated sediments (DiPinto & Coull, 1997). Given the weight of evidence to support direct ingestion as a principal route of contaminant exposure, it is possible the observed inhibition of EROD activity associated with direct contact is allied with a kinetic or temporal effect rather than bioavailability efficacy.

Interpretation of the screened contaminated sediment/normal diet versus contaminated sediment/normal diet treatment assumes contaminated Deceitful Cove sediment elicits a uniform induction rate throughout the duration of exposure. However, temporal fluctuations in hepatic EROD activity have been documented for several benthic fish species exposed to contaminated sediment (Collier & Varanasi, 1991; Payne *et al.*, 1988). Dab (*Limanda limanda*) directly exposed to PAH and PCB contaminated sediment exhibited a marked decline in EROD activity after an initial steep increase (Livingstone *et al.*, 1993). Whether EROD activity in non-screened contaminated sediment treatment within this study followed a similar time-course in inductive response is unknown. Conceivably, gross disturbance and direct physical contact with sediment in disturbed and non-screened contaminated treatments may have facilitated an expeditious transfer of contaminants from sediment to flounder, prompting earlier metabolisation of bioavailable organic compounds in these treatments. Further, "dredging" may have facilitated removal of some contaminants via resuspension and flowthrough seawater exchange. The time course of EROD activity varies with dosage and route of exposure (Peters *et al.*, 1997). The absence of a time-course analysis of EROD induction precludes any conclusion regarding contaminant uptake via screened verses direct contaminated sediment exposure, therefore the issue of possible temporal fluctuation in EROD activity of greenback flounder exposed to contaminated sediment from Deceitful Cove requires further investigation.

Notwithstanding the relatively short exposure of 6 weeks, the sub-lethal effects detected in growth and histological alteration of tissues are important in relation to the health of flounder exposed to contaminated sediments. These responses indicate that biochemical

alterations were sufficiently severe to lead to structural changes at the tissue level. If exposure to contaminated sediment in the field extends longer term, physiological impairment of the individual would most likely increase with increasing prevalence and severity of pathologic changes. Consequently, exposure to Deceitful Cove sediments may present a significant risk to flounder, particularly throughout the larval metamorphosis and juvenile stages. Juvenile greenback flounder predominantly congregate in shallow water with very fine to medium sandy substrates providing sheltered conditions and abundant suitable food supply (Crawford, 1984), and are therefore extremely vulnerable to anthropogenic disturbances. Cessation of contaminant exposure may enable subsequent recovery in adult flounder, known to occupy deeper water habitats within estuaries, provided tissue damage was not severe and contaminant exposure levels in deeper waters was below threshold levels.

Considering the evidence of organic contaminant uptake via diet in trial flounder, it is highly likely that xenobiotic uptake occurs in wild fish. The diet of greenback flounder is similar to that of sand flathead *Platycephalus bassensis* (Edgar, 1997). A recent field survey of sand flathead from Deceitful Cove found PCBs in skeletal muscle tissue of all individuals sampled (Mondon *et al.*, 1999c). Correlations between PCB, PAH and pesticide (DDT, DDD and DDE) levels and hepatic EROD response in sand flathead has been documented in laboratory and extensive field studies conducted in Port Phillip Bay (Holdway *et al.*, 1994; Brumley *et al.*, 1995). Given the results from the flounder trial and field survey, there is very strong evidence supporting bioaccumulation by benthic macroinvertebrates and transfer of contaminants to higher trophic levels in this region.

Limitations in extrapolating laboratory results to potential effects of contaminated sediments in the field are primarily restricted to the inability of the experimental flounder to avoid exposure to contaminated sediment and diet. Contaminant exposure was constant and as such modelled static contamination conditions in the field, with elevated turbidity and possible pulse exposure of sediment contaminants during gross disturbance.

Essentially, the disturbed contaminated sediment treatment represents the worst case scenario, potentially invalidated in the field due to the avoidance capability of wild fish. Furthermore, trial flounder were exposed to a fixed diet. It is possible that considerable variation exists in contaminant uptake via diet, depending on contaminant levels bioaccumulated by various benthic invertebrates in the field. Additionally, hatchery-reared flounder exhibited naive feeding behaviours compared to wild fish: hatchery flounder engulfed food in the manner of an ambush predator as it floated down from the surface, whereas wild greenback flounder actively dig for polychaetes and other invertebrates within the sediment (Edgar, 1997). Consequently, exposure to sediment contaminants via direct ingestion of sediments during feeding is potentially far higher for wild flounder in the field than those under trial conditions. Finally, extrapolation of greenback flounder response to contaminated sediment and diet to other teleost species may not be straightforward with respect to ecological niche and habitat. Benthic species with similar feeding habits and behaviours could be expected to exhibit similar responses. However, demersal species would be expected to exhibit a far greater histological response to sediment contamination than pelagic fish from the same region (Fournie *et al.*, 1996).

EROD, histological and growth biomarkers are of high toxicological relevance, and serve as indicators of marine sediment pollution at the biochemical, tissue and individual levels, respectively. However, impact of contaminated sediments at the population level is less clear. Consequently, investigation of longer term responses associated with reproduction and population growth would help to clarify causal relationships between chemical stressors at Deceitful Cove and their biological and ecological effect. In addition, validation of the laboratory-controlled biomarker assessment of Deceitful Cove sediments by field assessment will verify if short term biochemical and histological responses identified in this study are evident with *in situ* long term exposure.

This study supports the tenet of uptake of contaminant exposure by benthic teleost species as being primarily via direct contact with contaminated sediment and diet. Structural

evidence of multi-organ sensitivity to contaminated sediment from Deceitful Cove exists, suggesting these contaminants are cytotoxic. Organics, specifically CYP1A-inducing contaminants are present and bioavailable in Deceitful Cove. Histological alteration of the skin in flounder indicates a potentially significant impact of dredging operations on benthic teleost species inhabiting areas of contaminated sediments. Moreover, significant impairment of growth indicates possible reduced survival potential of wild flounder. Additional studies are required to further evaluate the responses of *R.tapirina* identified in this study as potentially effective biomarkers for assessment and monitoring of effects of marine pollution on benthic species in the field.

Acknowledgements

This study was funded by the Australian Research Council, TEMCO and Comalco Aluminium (Bell Bay) Limited. The authors thank Ms Carmel Pollino, Department of Applied Biology and Biotechnology, Royal Melbourne Institute of Technology, and Ms Jacqui Winterford, School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, for their technical assistence. Trace metal analyses of fish tissues and diets were performed by Dr Ashley Townsend, Central Science Laboratory, University of Tasmania. Organics analysis of fish tissues and diet were performed by the Tasmanian Department of Environment and Land Management Analytical Laboratory and Australian Environmental Laboratories, Hawthorn, Victoria.

References

Ankley, G. T., Schubauer-Berigan, M. K. & Dierkes, J. R. (1991). Predicting the toxicity of bulk sediments to aquatic organisms using aqueous test fractions: pore water verses elutriate. *Environmental Toxicology & Chemistry, 10*, 1359-1366.

Baker, J. T. P. (1969). Histological and electron microscopical observations on copper poisoning in the winter flounder *Pseudopleuonectes americanus. Journal of Fisheries Research Board Canada, 26*, 2785.

Brumley, C. M., Haritos, V. S., Ahokas, J. T. & Holdway, D. A. (1995). Validation of biomarkers of marine pollution exposure in sand flathead using Aroclor 1254. *Aquatic Toxicology, 31*, 249-262.

Burke, M. D. & Mayer, R. T. (1974). Ethoxyrufin: direct flourimetric assay of a microsomal)-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metabolism &Disposition, 2*, 583-588.

Chapman, P. M. (1990). The Sediment Quality Triad approach to determining pollution-induced degradation. *Science of the Total Environment, 97/98*, 815-825.

Collier, T. K. & Varanasi, U. (1991). Hepatic activities of xenobiotic metabolising enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. *Archives of Environmental Contamamination &Toxicology, 20*, 462-473.

Courtney, W. A. M. (1980). Accumulation of polychlorinated biphenyls in turbot (*Scolphthalmus maximus*) from sea water sediments and food. *Helgolander Meeresunters, 33*(333-339).

Crawford, C. M. (1984). *An ecological study of Tasmanian Flounder., PhD.Thesis.* , University of Tasmania, Hobart.

Depledge, M. H. (1993). The rational basis for the use of biomarkers as ecotoxicological tools. In M. C. Fossi & C. Leonzio (Eds.), *Non-destructive biomarkers in vertebrates* . Boca Raton: Lewis publishers.

Depledge, M. H., Aagaard, A. & Gyorkos, P. (1995). Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. *Marine Pollution Bulletin, 31*(1-3 Special issue SI), 19-27.

DiPinto, L. M. & Coull, B. C. (1997). Trophic transfer of sediment-associated polychlorinated biphenyls from meiobenthos to bottom-feeding fish. *Environmental Toxicology and Chemistry, 16*(12), 2568-2575.

Edgar, G. J. (1997). *Australian Marine Life the plants and animals of temperate waters*. Kew: Reed Books.

Fournie, J. W., Summers, J. K. & Weisberg, S. B. (1996). Prevalence of gross pathological abnormalities in estuarine fishes. *Transactions of American Fisheries Society, 125*(4), 581-590.

Heath, A. G. (1987). *Water pollution and fish physiology*. Boca Raton: CRC Press.

Hinton, D. E. & Lauren, D. J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: Potential biomarkers of exposure. In J. F. McCarthy & L. R. Shugart (Eds.), *Biomarkers of environmental contamination* (pp. 17-57). Boca Raton: CRC Press.

Holdway, D. A., Brennan, S. E. & Ahokas.J.T. (1994). Use of hepatic MFO and blood enzyme biomarkers in sand flathead (*Platycephalus bassensis*) as indicators of pollution in Port Phillip Bay, Australia. *Marine Pollution Bulletin, 28*(11), 683-695.

Lindesjoo, E., Husoy, A. M., Petterson, I. & Forlin, L. (1996). Histopathological and immunohistochemical studies in roundnose grenadier (*Coryphaenoides rupestris*) in the Skagerrak, North Sea. *Marine Environmental Research, 4*(1-4), 229-233.

Livingstone, D. R., Lemaire, P., Mattews, A., Peters, L., Bucke, D. & Law R, J. (1993). Pro-oxidant, anti-oxidant and 7-ethoxyreorfin o-deethylase (EROD) activity responses in liver of Dab (*Limanda limanda*) exposed to sediment contaminated with hydrocarbons and other chemicals. *Marine Pollution Bulletin, 26*(11), 602-606.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with Folin phenol reagent. *J.Biol.Chem., 193*, 265-275.

Matthiessen, P. & Brafield, A. E. (1973). The effects of dissolved zinc on the gills of the stickleback, *Gasterosteus aculeatus* (L). *Journal of Fish Biology, 5*, 607.

Mondon, J., Richardson, A. M. M. & Nowak, B. F. (1999a). Sediment Quality Triad assessment of trace metals and organic contamination of shallow subtidal marine sediments near a ferro-manganese smelter and aluminium refining plant. *Under review.*

Mondon, J. A., Duda, S. & Nowak, B. F. (1999b). Immune response of contaminated marine sediment and diet on Greenback flounder *Rhombosolea tapirina. Marine Environmental Research (Accepted for publication).*

Mondon, J. A., Nowak, B. F. & Sodergren, A. (1999c). Persistent organic pollutants in oysters *Crassostrea gigas* and sand flathead *Platycephalus bassensis* from Tasmanian estuarine and coastal waters. *Marine Pollution Bulletin (Accepted for publication).*

Murty, A. S. (1985). *Toxicity of pesticides to fish.* (Vol. 2): CRC Press.

Oronsaye, J. A. O., & Braefield, A. E. (1984). The effect of dissolved cadmium on the chloride cells of the gills of the stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology, 25*, 253.

Pascoe, G. A., Blanchet, R. J., Linder, G., Palawski, D., Brumbaugh, W. G., Canfield, T. J., Kemble, N. E., Ingersoll, C. G., Farag, A. & Dalsoglio, J. A. (1994). Characterization of ecological risks at the Milltown Reservoir Clark Fork River Sediments Superfund Site. *Environmental Toxicology & Chemistry, 13*(12), 2043-2058.

Pawert, M., Muller, E. & Triebskorn, R. (1998). Ultrstructural changes in fish gills as biomarker to assess small stream pollution. *Tissue & Cell, 30*(6), 617-626.

Payne, J. F., Kiceniuk, J., Fancey, L. L., Williams, U., Fletcher, G. L., Rahimtula, A. & Fowler, B. (1988). What is a safe level of polycyclic aromatic hydrocarbons for fish: subchronic toxicity study on winter flounder (*Pseudopleuronectes americanus*). *Canadian Journal of Fisheries & Aquatic Sciences, 45*, 1983-1993.

Peters, L. D., Morse, H. R., Waters, R. & Livingstone, D. R. (1997). Responses of hepatic cytochrome P450 1A and formation of DNA-adducts in juveniles of turbot (Scophthalmus maximus L.) exposed to water-borne benzo(a)pyrene. *Aquatic Toxicology, 38*, 67-82.

Peters, L. D., O'Hara, S. C. M. & Livingston, D. R. (1996). Benzo(*a*)pyrene metabolism and xenobiotic-stimulated reacyive oxygen species generation by subcellular fraction of larvae

of turbot (*Scophthalmus maximus* L.). *Comparative Biochemistry & Physiology, 114C*(3), 221-227.

Powell, M. D., Wright, G. M. & Speare, D. J. (1995). Morphological changes in rainbow trout (*Oncorhynchus mykiss*) gill epithelia following repeated intermittent exposure to chloramine-T. *Canadian Journal of Zoology, 73*(154-165).

Powell, M. D. & Perry, S. F. (1997). Respiratory and acid-base pathophysiology of hydrogen peroxide in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquatic Toxicology, 37*, 99-112.

Shephard, K. L. (1994). Functions for fish mucus. *Reviews in Fish Biology and Fisheries, 4*, 401-429.

Stegeman, J. J. & Lech, J. J. (1991). Cytochrome p-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environmental Health Perspectives, 90*, 101-109.

Taveekijakarn, P., Miyazaki, T., Matsumoto, M. & Arai, S. (1996). Histopathological and haematological changes in amago salmon, *Oncorhynchus rhodurus* (Jordan-and McGregor), fed a vitamin-D-free diet. *Journal of Fish Diseases, 19*(4), 289-294.

Thiyagarajah, A., Hartley, W. R., Major, S. E. & Broxson, M. W. (1996). Gill histology of two species of buffalo fish from a contaminated swamp. *Marine Environmental Research, 42*(1-4), 261-266.

Underwood, A. J. (1981). Techniques of analysis of variance in experimental marine biology and ecology. *Oceanography & Marine Biology Annual Review, 19*, 513-605.

van der Weiden, M. E. J., Tibosch, H. J. H., Bleumink, R. & Sinnige, T. L. (1993). Cytochrome P450 1A in the common carp (*Cyprinus carpio*) following exposure to contaminated sediments with halogenated polyaromatics. *Chemosphere, 27*(7), 1297-1309.

Woodward, D. E., Farag, A. M., Bergman, H. L., Delonay, A. J., Little, E. E., Smith, C. E. & Barrows, F. T. (1995). Metals-contaminated benthic invertebrates in the Clark Fork River, Montana - effects on age 0 brown trout and rainbow trout. *Canadian Journal of Fisheries & Aquatic Sciences, 52*(9), 1994-2004.

Vethaak, A. D., Jol, J. G., Meijboom, A., Eggens, M. L., Aprheinallt, T., Wester, P. W., Vandezande, T., Bergman, A., Dankers, N., Ariese, F., Baan, R. A., Everts, J. M., Opperhuizen, A. & Marquenie, J. M. (1996). Skin and liver diseases induced in flounder (*Platichthys flesus*) after long-term exposure to contaminated sediments in large-scale mesocosms. *Environmental Health Perspectives, 104*(11), 1218-1229.

Vethaak, A. D. & Wester, P. W. (1996). Diseases of flounder Platichthys flesis in Dutch coastal and estuarine waters, with particular reference to environmental stress factors. 2. Liver histopathology. *Diseases of Aquatic Organisms, 26*(2), 99-116.

CHAPTER FIVE

Immune response of Greenback flounder *Rhombosolea tapirina* after exposure to contaminated marine sediment and diet

J.A. Mondon[1] S. Duda[2] and B.F.Nowak[1]

1 School of Aquaculture, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, P.O.Box 1214, Launceston, Tasmania 7250, Australia.
2 Key Centre for Applied and Nutritional Toxicology, Royal Melbourne Institute of Technology-University, GPO Box 2476V, Melbourne 3001, Victoria, Australia.

Accepted for publication in *Marine Environmental Research*

ABSTRACT

Non-specific immune response of Greenback Flounder, *Rhombosolea tapirina*, exposed to contaminated marine sediments was examined. Reference sediments from Port Sorell and contaminated sediments from Deceitful Cove, Tasmania, Australia were investigated. Hatchery-reared flounder were exposed to reference sediment, contaminated sediment or contaminated sediment and diet for 6 weeks. Phagocytic capacity and lysozyme response in flounder were examined on cessation of exposure trial. Significant differences were found in phagocytic capacity and lysozyme response between treatments. Exposure to contaminated sediment, irrespective of diet or benthic disturbance elicited inhibition of phagocytic efficiency in flounder. Disturbance of contaminated sediment stimulated lysozyme activity. The immune response in flounder indicates potential immunotoxicity of sediment from Deceitful Cove.

Introduction

The presence of heavy metals and aromatic organic compounds in the aquatic environment may adversely affect fish immune response (for review see Anderson & Zeeman,1995). Exposure to immunotoxicants can result in increased susceptibility to disease (Weeks *et al.*, 1992) the implications of which can impact at the individual, population and possibly community level (Hartwell *et al.*, 1992; Landahl *et al.*, 1997).

Screening of non-specific immune responses provides a first tier evaluation of potential immunotoxicity in higher level aquatic organisms. Of the wide range of immune assays available phagocytic capacity, an important component of the innate immunodefense system and lysozyme response enable expeditious evaluation of immunotoxicity at the cellular and humoral level.

Evidence of contamination of shallow subtidal marine sediments and toxicity at the unicellular organism level in Deceitful Cove, Tasmania, has been established (Table 1). However, impact at higher organism levels is unknown and extrapolation from invertebrate toxicity data to vertebrates is difficult. There is a paucity of research dealing with effects of contaminated sediment on fish and an absence of sediment toxicity data for Australian native fish. Furthermore, remediation of sediments by dredging has been proposed for the bay. Whether or not dredging will increase toxicity of deposited contaminants is unknown.

Table 1. Mean (± S.E.) values of pooled porewater toxicity data for Microtox® (marine bacterium) and *Nitzchia closterium* (marine diatom) bioassays (1996-1997). Light reduction emission levels above 0% indicate increasing levels of toxicity. EC_{50} growth inhibition levels less than 100 indicate increasing levels of toxicity. Different superscript letters indicate statistically significant differences ($p<0.05$) within each bioassay.

Bioassay	Deceitful Cove (contaminated)	Squeaking Point (reference)
Microtox® Medical Device Screening Protocol		
% light reduction emission	26.96 ± 1.28 [a]	0 [b]
Nitzchia closterium		
EC_{50} growth inhibition	41.30 ± 3.54 [a]	>100 [b]

Toxicity of chemicals is usually evaluated in aqueous solution. However, the main route of exposure for benthic species is often via contact with contaminated sediment and diet (for review see Chapman *et al.* 1998). The primary aim of this study is to assess the effects of contaminated sediment, diet and disturbance on the immune response of greenback flounder, and to provide background data from which to assess remediation of sediment in the future.

Table 2. Mean (± S.E.) values for selected geochemical parameters of pooled shallow subtidal sediment data from Deceitful Cove and Port Sorell (1996-1997).

Parameter	Deceitful Cove (contaminated)	Squeaking Point (reference)
Granulometry		
Wentworth classification	medium to fine sand	medium to fine sand
% mud (silt + clay)	12.84 ± 0.5	12.83 ± 0.42
%TOC	16.4 ± 0.1.19	7.04 ± 0.18
Trace metals	(*mg kg^{-1} dry wt.*)	(*mg kg^{-1} dry wt.*)
Ag	0.18 ± 0.04	0.04 ± 0.008
Al	12660.8 ± 2643	3217.7 ± 156.7
Cd	1.93 ± 0.49	0.06 ± 0.01
Cr	14.6 ± 1.14	10.44 ± 0.32
Cu	26.83 ± 3.1	3.8 ± 1.25
Fe	11195.7 ± 840.2	6485.2 ± 272.5
Mn	57215.9 ± 10808	45.1 ± 2
Ni	75.1 ± 20.86	3.07 ± 0.236
Pb	322.28 ± 68.54	8.4 ± 1.75
Zn	1064.18 ± 235.16	10.39 ± 0.98
PAHs	(*µg kg^{-1} dry wt.*)	
Benzo(*a*)anthracene	916.67 ± 279.6	nd
Benzo(*b*)fluoranthene	1183.0 ± 263.11	nd
Benzo(*k*)fluoranthene	375.0 ± 95.4	nd
Benzo(*a*)pyrene	783.3 ± 180.34	nd
Benzo(*ghi*)perylene	191.67 ± 88.65	nd
Chrysene	454.2 ± 159.03	nd
Fluoranthene	2041.0 ± 711.29	nd
Indeno(*1,2,3-cd*)pyrene	179.17 ± 77.55	nd
Phenanthrene	225.0 ± 100.3	nd
Pyrene	1445.83 ± 425.16	nd

nd = not detected

Methods

Hatchery-reared flounder were exposed to shallow sub-tidal sediment from contaminated and non-contaminated (reference) estuarine sites. Dredging of sediment was simulated under laboratory conditions, and the impact of exposure to diet and sediment from the contaminated site evaluated.

Twelve month old greenback flounder (44.31 ± 2.9 g (standard error) individuals) were acclimated to 15-20 ppt salinity for two weeks at 14°C under 12:12 hour light dark regime and fed with flounder pellets. Post acclimation the fish were divided into 5 experimental treatment groups:

1. exposure to reference sediment and uncontaminated feed
2. exposure to screened contaminated sediment and uncontaminated feed
3. exposure to disturbed contaminated sediment and uncontaminated feed
4. exposure to contaminated sediment and uncontaminated feed
5. exposure to contaminated sediment and contaminated feed

Each replicated experimental treatment comprised of 10 fish per 250 L tank, flow-through, aerated, in-line 5 μm filtered (Cuno Water Filter System) seawater (15-20 ppt), plus eight litres of sediment. Contaminated (Deceitful Cove) and reference (Port Sorell) sediments were selected based on geochemical data obtained during the 1996-1997 survey (Table 2). Sediment from the field was collected using 70 mm diameter benthic core tubes (to minimise surficial disturbance), inserted 10 cm into sediments. Samples were pooled and stored in sealed, cooled, acid washed food grade containers for immediate transportation to the laboratory. Sediment was transferred to treatment tanks containing fresh seawater to a depth of 10 cm and allowed to settle before the tanks were trickle filled. A 5 mm mesh was placed on top of the sediment in conditions 1, 2, 4 & 5 to reduce turbidity of resuspended sediment whilst allowing direct contact between sediment and fish during feeding and rest. A 0.25 mm mesh was used to inhibit direct contact with sediment for experimental condition 3. Simulated dredging activity in treatment 4 involved moving the mesh to one

side of the tank and scooping sediment up by hand and releasing it at the water surface repeatedly for 10 minutes, twice daily.

All fish were fed approximately 4.2 g feed/fish/day (dry weight), approximately 9% body weight/day. Feed comprised of a homogenised mixture of 1:30:30 gelatine, flounder pellets and farmed oyster meat (uncontaminated diet) or oyster meat collected from Deceitful Cove (contaminated diet). Diets were analysed for trace metals and organic contaminant concentrations. Diets were oven dried overnight at 70°C, ground, digested in nitric acid and analysed by ICP-Optical Emission Spectrometer (ICP-OES) for trace metals, detection limit 0.1 mg kg^{-1} dry weight (Table 3). For PAH detection, diet samples were mixed with sodium sulphate and crushed, dichloromethane/acetone was then added, the samples sonicated and shaken, and the extract analysed by gas chromatography - mass spectrum (GC - MS), detection limit 0.2 mg kg^{-1} dry weight (Table 3).

After a 6 week exposure trial the fish were anaesthetised with benzocaine (50 ppm) and blood collected from the caudal vein, then euthanised (benzocaine 150 ppm) without recovery and the head kidney removed. Two non-specific immune defences were assessed: lysozyme activity and phagocytic efficiency.

Lysozyme activity was determined spectrophotometrically. A suspension of *Micrococcus lysodeikticus* (0.75 mg ml^{-1}) was prepared in 0.1M phosphate buffer (pH 5.8). Flounder blood plasma (0.25 ml) was added to *Micrococcus* suspension (175 ml) and the decrease in absorbence recorded at 450 nm over 5min. Hen egg white lysozyme standards were used to convert lytic activity into lysozyme concentrations.

The phagocytic function of flounder macrophages was determined by microscopic measurement of the number of congo red-stained yeast cells phagocytized per macrophage, using the method of Seeley *et al.* (1990). After aseptic removal from flounder, the head kidney was placed into petrie dishes containing 4 mL phosphate buffered saline (PBS) pH 7.4. Single cell suspensions were made by gently drawing kidney through a 2 mL syringe and filtered over a nylon mesh funnel into 10 mL centrifuge tubes. The suspension was

washed three times in 8 mL PBS. Supernatant was removed and the pellet made up to 4 mL with PBS. Congo red stained yeast cells, *Saccromyces cerevisciae*, (200 µL of a 108 cells mL^{-1} suspension) were added to tubes and mixed by hand. Tubes were then centrifuged to 600 rpm for 30 minutes at 15°C. Supernatant was removed and 4 mL ice cold PBS was added. The suspension was carefully layered on 3 mL Ficoll-Paque (Sigma). Tubes were then centrifuged at 1500 rpm for 20 minutes at 4°C. After centrifugation the interface layer was removed and washed in PBS, with the pellet being made up to 1 mL PBS. Phagocytosis was evaluated by counting the number of yeast cells per phagocyte from smears prepared for each sample. One hundred phagocytes were counted per slide (400x magnification). Data for both assays were analysed using nested ANOVA.

Table 3. Trace metals and PAH levels detected in contaminated and non-contaminated flounder diets.

Contaminant	Detection limit	Contaminated diet	Uncontaminated diet
Trace metals (mg kg^{-1} dry wt.)	**(ppm)**		
Al	0.1	107.47	35.46
As	0.1	3.77	2.46
Cd	0.1	3.98	1.96
Cr	0.1	1.41	0.37
Cu	0.1	245.06	22.375
Fe	0.1	336.86	262.56
Mn	0.1	105.45	80.52
Ni	0.1	0.86	0.18
Pb	0.1	1.7	<0. 1
Sn	0.1	8.45	6.62
Zn	0.1	1114	248.65
PAHs (mg kg^{-1} dry wt.)			
Acenaphthylene	0.2	nd	nd
Anthracene	0.2	nd	nd
Benzo(*a*)anthracene	0.2	0.2	nd
Benzo(*b*)fluoranthene	0.2	nd	nd
Benzo(*k*)fluoranthene	0.2	nd	nd
Benzo(*a*)pyrene	0.2	0.3	nd
Benzo(*ghi*)perylene	0.2	nd	nd
Chrysene	0.2	0.2	nd
Dibenzo(*ah*)anthracene	0.2	nd	nd
Fluoranthene	0.2	0.4	nd
Fluorene	0.2	nd	nd
Indeno(*123-cd*)pyrene	0.2	nd	nd
Naphthalene	0.2	nd	nd
Phenanthrene	0.2	nd	nd
Pyrene	0.2	0.6	nd

nd = not detected

Results and discussion

A significant difference in lysozyme response was demonstrated in flounder exposed to disturbed sediment, the mean lysozyme concentration induced by disturbed sediment averaging twice the concentration of other treatments (Figure 1*a*).

Phagocytic efficiency was significantly different between reference and contaminated sediment with a reduction in capacity when exposed to contaminated diet. There was a trend towards decreasing efficiency with direct exposure to sediment culminating in lowest efficiency associated with contaminated diet. However, comparisons of screened contaminated sediment and uncontaminated diet vs contaminated sediment and uncontaminated diet, disturbed contaminated sediment and uncontaminated diet vs undisturbed contaminated sediment and uncontaminated diet, and contaminated sediment and contaminated diet vs contaminated sediment and uncontaminated diet resulted in non-significant differences (Figure 1*b*), most likely due to high variation between tanks.

The results indicate that exposure to contaminated sediment, irrespective of diet or benthic disturbance, elicits an inhibition of phagocytic efficiency in flounder, whereas disturbance of contaminated sediment stimulates lysozyme activity. Whether the stimulus of lysozyme activity was elicited by the physical disturbance of the benthos or by exposure to higher levels of contaminants via resuspension, or both, is not clear. Furthermore, due to the complex nature of the contaminants the pollutant(s) potentially responsible for phagocytosis immunosuppression are unknown: positive identification would require extensive toxicity identification evaluation (TIE).

Complex contaminant exposure and physical stress have been linked to enhanced lysozyme activity (Secombes *et al.*, 1995; Demers& Bayne,1997). Similarly, suppression of phagocytosis efficiency has also been documented in fish from known contaminated sites, including the common toadfish *Opsanus tau* and the goby *Zosterisessor ophiocephalus* (Seeley & Weeks-Perkins,1991; Pulsford *et al.*, 1995a).

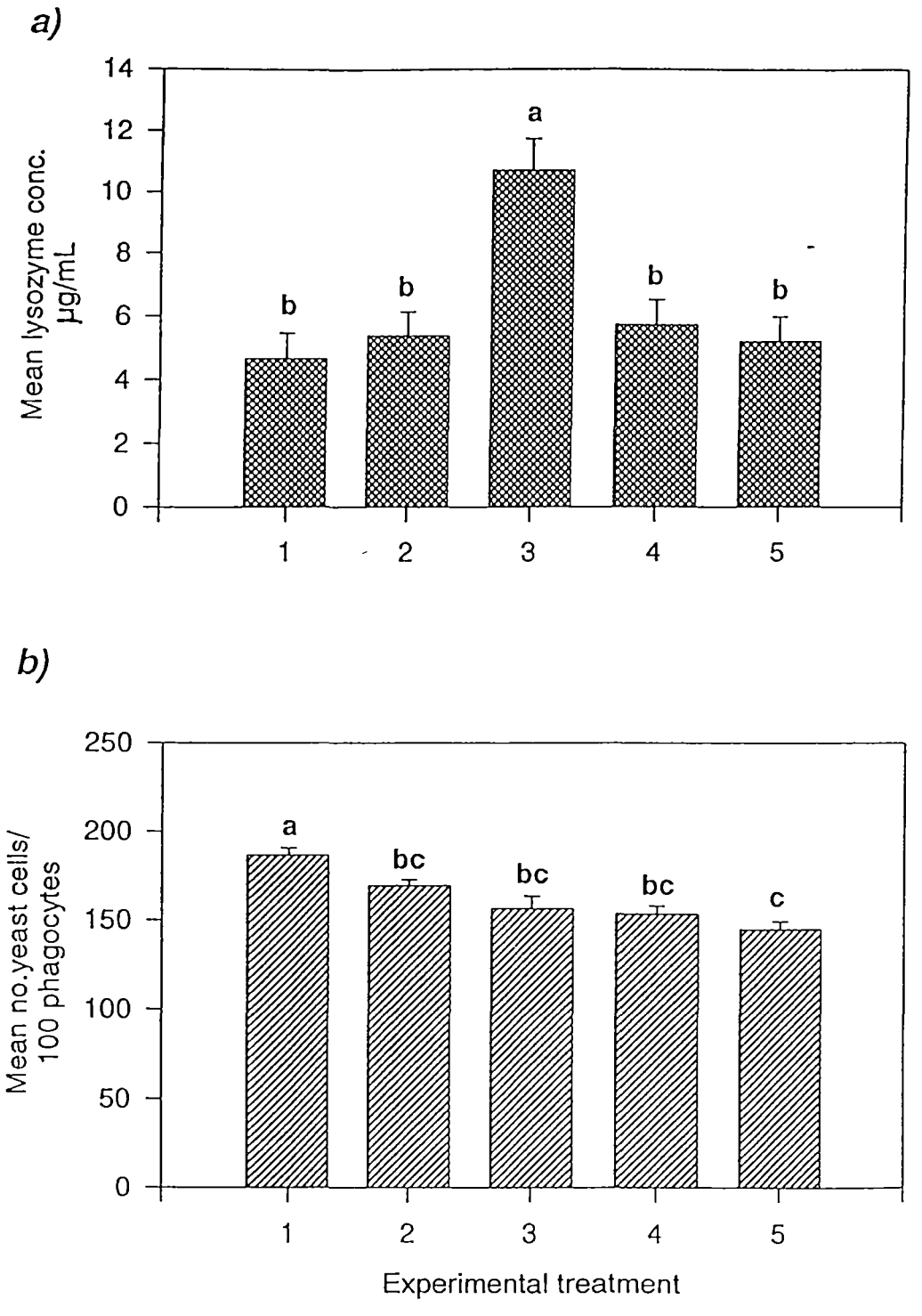


Figure 1. **Mean (± S.E.) values of lysozyme concentrations in response to contaminated sediment in greenback flounder *Rhombosolea tapirina*: a) lysozyme concentration, b) phagocytic capacity, 1. reference sediment + uncontaminated food, 2. screened contaminated sediment + uncontaminated food, 3. disturbed contaminated sediment + uncontaminated food 4. contaminated sediment + uncontaminated food 5. contaminated sediment + contaminated food. Different letters indicate statistically significant differences ($p<0.05$).**

Stimulatory and suppressive variation between immunological parameters in fish is not uncommon (Anderson & Zeeman,1995; Pulsford *et al.*,1995a; Pulsford *et al.*, 1995b). Even though chronic stress from contaminants will eventually be immunosuppressive (Anderson & Zeeman, 1995), the acute stress from simulated dredging may have enhanced the humoral components of innate defences to ameliorate the flounders' capability to cope with a chronically stressful environment (Demers & Bayne, 1997). Physical disturbance of the sediment, rather than elevated contaminant exposure, may be the basis for the increased lysozyme levels as lyzozyme is thought to be a defence against microbes, most likely resuspended along with other fine particulate matter during dredging.

Considering the relatively short-term exposure to contaminated sediment, it can reasonably be inferred that the observed immune response presented in flounder indicates potential immunotoxicity of sediment in the Deceitful Cove region. These findings necessitate further investigation of the sediment utilising a broader suite of immunoassays incorporating disease challenge, indicating the ability of the immune response in benthic fish to cope with pathogens, and field validation. Furthermore, the results emphasise the need to employ a number of immunassays in evaluation of contaminated sites.

Acknowledgement - This study was funded by the Australian Research Council, TEMCO and Comalco Aluminium (Bell Bay) Limited. Trace metal analyses of sediments and diets were performed by Dr Ashley Townsend, Central Science Laboratory, University of Tasmania. Organics analysis of sediments and diets were performed by Australian Environmental Laboratories, Hawthorn, Victoria.

References

Anderson, D. P. & Zeeman, M. G. (1995). Immunotoxicology in fish. In G. M. Rand (Ed.), *Fundamentals of aquatic toxicology* (pp. 371-404). Washington: Taylor & Francis.

Chapman, P.M., Wang, F., Jannsen, C., Persoone, G. & Allen, H. (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability, risk assessment, and remediation. *Canadian Journal of Fisheries and Aquatic Sciences, 55,* 2221-2243.

Demers, N. E. & Bayne, C. J. (1997). The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. *Developmental & Comparative Immunology, 21*(4), 363-373.

Hartwell, S. I., Dawson, C. E., Durell, E. O., Alden, R. W., Adolphson, P. C., Wright, D. A., Coelho, G. M., Magee, J. A., Ailstock, S. & Norman, M. (1997). Correlation of measures of ambient toxicity and fish community diversity in Chesapeake Bay, USA, tributaries- urbanizing watersheds. *Environmental Toxicology & Chemistry, 16*(12), 2556-2567.

Landahl, J. T., Johnson, L. L., Stein, J. E., Collier, T. K. & Varanasi, U. (1997). Approaches for determining effects of pollution on fish populations of Puget Sound. *Transactions of the American Fisheries Society, 126*(3), 519-535.

Pulsford, A. L., Thomas, M. E., Lemaire-Gony, S., Coles, J., Fossato, V. U. & Pipe, R. K. (1995a). Studies on the immune system of the Goby, *Zosterisessor ophiocephalus*, from the Venice Lagoon. *Marine Pollution Bulletin, 30*(9), 586-591.

Pulsford.A.L., Crampe, M., Langstone, A. & Glynn, P. J. (1995b). Modulatory effects of disease, stress, copper, TBT and vitamin E on the immune system of flatfish. *Fish and shellfish immunology, 5*, 631-643.

Secombes, C. J., White, A., Fletcher, T. C., Stagg, R. & Houlihan, D. E. (1995). Immune parameters of plaice, *Pleuronectes platessa*, along a sewage sludge gradient in the Firth of Clyde, Scotland. *Ecotoxicology, 4*(5), 329-340.

Seeley, K.R., Gillespie P.D. & Weeks, B.A. (1990). A simple technique for the rapid spectrophotometric determination of phagocytosis by fish macrophages, *Marine Environmental Research, 30*, 37-41

Seeley, K. R. & Weeks-Perkins, B. A. (1991). Altered phagocytic activity of macrophages in Oyster Toadfish from a highly polluted subestuary. *Journal of Aquatic Animal Health, 3*, 224-227.

Weeks, B. A., Anderson, D. P., DuFour, A. P. & Peters, G. (1992). Immunological biomarkers to assess environmental stress. In R. J. Huggett, R. A. Kimerle, P. M. J. Mehrke & H. L. Bergman (Eds.), *Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress.* Boco Raton: Lewis.

CHAPTER SIX

General Discussion

Successful adaptation of standard marine water toxicity bioassays to assess porewater toxicity enabled this study to establish clear relationships between toxicity, chemical composition and benthic communities of shallow subtidal sediments at contaminated and non-contaminated sites in northern Tasmania. The liquid phase Microtox® and algal growth bioassays are suitable for testing porewater toxicity of coastal marine sediments. However, difficulty in interpreting Microtox® solid phase test results limits the use of this assay for routine testing (Appendix 1). Additionally, the sea urchin *Heliocidaris tuberculata* bioassay, which exhibits elevated sensitivity to porewater contaminants, requires further research on extending the spawning period of this species, before *H. tuberculata* can be used for routine bioassay work in southern temperate Australian coastal waters.

The correspondence between sediment chemistry, toxicity and field effects in the sediment quality triad analysis was used in conjunction with the laboratory based assessment of contaminated sediment, diet and re-suspension of sediment on benthic finfish, to determine chemical concentrations in sediment that discriminated conditions of minimal, uncertain and potentially major biological effects in the Tamar River estuary.

Previous studies evaluating chemical contamination of Deceitful Cove assessed bulk sediment chemistry and bioaccumulation of metals and PAHs in oyster tissue *Crassostrea gigas* (Gawne & Richardson, 1992; Miedecke, 1992a), and to a very limited extent sea lettuce (possibly *Ulva sp.*), crabs (unidentified) and flathead *Platycephalus bassensis* (Miedecke, 1992b). Both investigations concentrated predominantly on the impact of the contamination with respect to human consumption of contaminated organisms. In this study, the effect of sediment contamination was investigated to determine the retrospective and future ecological impact of contaminants in Deceitful Cove, and the lower Tamar River estuary.

The shallow subtidal sediments of Deceitful Cove are a chemically-stressed environment, where, out of a range of contaminants, trace metals were predominantly linked to changes in benthic macroinvertebrate assemblage patterns, possibly due to toxicity associated with the chemicals present (Chapter 1). Sediment toxicity is not isolated to the Deceitful Cove region of the Tamar River estuary. East Arm also exhibits evidence of environmental stress associated with sediment contaminants (Chapter 1).

Direct contact with contaminated sediment and diet, and re-suspended contaminated sediments resulted in uptake of contaminants by benthic fish (Chapter 3). Investigation of the non-specific biomarker response of greenback flounder indicated that Deceitful Cove sediment exhibits potential immunotoxicity and cytotoxicity properties (Chapter 4). Results suggest that *in situ* exposure to contaminated sediment and diet, may elicit a multi-organ histological response, growth inhibition, suppressed non-specific immune response, and uptake of CYP1A inducing compounds in wild benthic fin-fish. Additionally, disturbance of Deceitful Cove sediment presents a potential risk associated with ingestion of CYP1A inducing contaminants bound to the re-suspended sediment particles (Chapter 3). Increased exposure to pathogens via sediment re-suspension may further compromise the health of finfish if, as the study suggests, their immune system is suppressed by chemical or physical stress (Chapter 4).

Seasonal PAH contamination of Deceitful Cove sediments is apparent, in addition to high trace metal concentrations, compared to locations where industrial activity is absent. There is also evidence of bioaccumulation of organochlorines in the muscle tissue of benthic fin-fish and shellfish from the Deceitful Cove area (Mondon *et al.*, 1999, Appendix 5), although the source and spatial bioavailability of these compounds in the lower Tamar River region has not been established.

The proposal to dredge contaminated sediments from Deceitful Cove requires careful consideration. Dredging enables rapid whole scale removal of contaminated sediment, but at the same time runs the risk of re-suspending not only the buried anthropogenic pollutants, but also naturally occurring toxicants such as NH_4 and H_2S that may be enriched

in polluted sediments (Peddicord *et al.*, 1995). A marked increase in the turbidity of the water column is also likely to occur (Chapman *et al.*, 1998). Removal of the benthic macroinvertebrate habitat would result in a reduction in the trophic transfer of contaminants from invertebrates to benthic finfish (Peddicord *et al.*, 1995). However, dredging of sediments may also increase the direct exposure to pollutants to finfish via ingestion of resuspended contaminant bound particles (Chapter 3).

The results of this study indicate that the sediments of Deceitful Cove are contaminated and remediation is warranted. Nine ER-M sediment quality guideline (SQG) values were exceeded indicating the chemical concentrations present in the sediment would be highly likely to cause harm (Chapter 2). However, it should be noted that the SQG values used have not yet been field validated for Australian coastal waters. Additionally, the aggressive acid digestion technique used in measuring bulk metal chemistry may result in levels that over-estimate risk, by releasing elements bound in insoluble form, which are thus not bioavailable (Chapman, 1990). Only the biologically available fraction is relevant to the actual contaminant exposure that may ultimately elicit adverse ecosystem impacts (Peddicord *et al.*, 1995).

Application of the sediment quality triad (SQT), in conjunction with SQGs, indicated strong evidence of contaminant-induced stress, and possible environmental degradation at Deceitful Cove (Chapter 1). However, the degree of chemical stress or degradation is unknown as the investigation was limited by the absence of *a priori* data on species assemblage and toxicity. Results suggest that there has been a moderate impact of contamination on macroinvertebrate assemblages (Chapter 2), relative to the anticipated toxicity indicated by bioassay results (Chapter 1). However, the altered community structure is still likely to be a response of resident populations to chemical stress (Attril & Depledge, 1997), given that the species assemblages in the vicinity of Deceitful Cove were different from those in similar habitats remote from industry. Since interacting geochemical reactions and biological processes can change pollutant form and bioavailability as ecosystem conditions change over time and space, the macroinvertebrate

- the reduction in direct chemical input from the Bell Bay industrial estate over recent years,
- generational times of macroinvertebrates may be measured in years, so that a stable community characterising the new environmental conditions (ie. reduced chemical input) may take several years to establish (or stabilise) (Underwood, 1996), and
- that the community assemblage in Deceitful Cove may be a response to earlier contamination deposited prior to the recent reduction in chemical input.

Athough the study identified a lower species diversity at Deceitful Cove relative to reference locations, the species present at Deceitful Cove represent a broad functional and taxonomic diversity (Appendix 4b). Additionally, the absence of known indicator species, which tend to dominate species assemblages under severe pollution (Gray *et al.*, 1990), lends further weight to the hypothesis that deleterious contaminant impact at Deceitful Cove is not extreme. A definitive interpretation of the response of macrobenthos to environmental conditions at Deceitful Cove would require field experimentation, involving multi-species community level testing.

The assessment of the effect of contaminant exposure on greenback flounder assumed that metal speciation over the duration of the exposure was uniform, and the conditions governing metal speciation *in situ* were replicated under laboratory conditions. However, field conditions can differ markedly from the laboratory (Chapman, 1995). *In situ* sediment represents an infinitely larger, more dynamic, interactive system than the highly simplified laboratory habitat. The spatial and temporal variability of bioavailability that occurs in the field cannot be simulated exactly in the laboratory (Underwood, 1996; Chapman *et al.*, 1998). Sediment characteristics, such as TOC and acid volatile sulphides, and changes in pH and redox potential which affect the complexing and solubility of trace metals, in addition to biotic factors such as bioturbation, all significantly influence the bioavailability of contaminants, and are not static variables. (Chapman *et al.*, 1998). Consequently, the experimental procedure may not mimic the exact exposure conditions *in situ*, but instead represents a temporal snapshot of the effects of a representative set of environmental conditions likely to occur in the field. Laboratory-based exposures also

exhibit a tendency to over-estimate the bioavailability of pollutants *in situ* (Chapman, 1995), particularly when, as in this case, the experimental fish were unable to modify their exposure by avoidance or variation in diet. Hence, the results are indicative of a worse case.

Combined laboratory and field biomarker screening of benthic finfish are therefore necessary to strengthen the basis for extrapolation from laboratory data to actual impact in the field. Contaminant impact was evident under laboratory conditions, with measurable signals of changes at the biochemical, cellular and individual level. However, although induced physiological impairment has been related via physiological costs to fitness of the individual (Luoma, 1996), this could not be extended to providing information on the reproductive efficiency of individuals and populations exposed to contaminants. Inherent differences in sensitivity to pollution among individuals from wild fish populations may reduce the potential impact of contaminants in the field (Luoma, 1996). However, the costs of surviving pollutant exposure via physiological compensation could result in impairment of processes important to the success of the population, for example, through reduced fecundity (Silby *et al.*1989). Furthermore, these populations may be less able to adapt to additional environmental changes or stresses (Klerks & Weis, 1987). Investigation of field populations of benthic finfish is necessary to determine whether the effects evident in the laboratory are present in the field. Because of the mobility of benthic fish relative to macroinvertebrate assemblages, greenback flounder, and other benthic fish species, would provide a regional rather than localised perspective on the geographical extent of bioavailability and contaminant impact (Warwick, 1993).

This is the first published sediment quality triad (SQT) study in Australia, and as with northern hemisphere studies, highlights the limitations associated with relying solely on bulk sediment chemistry or community structure to assess sediment quality. The combination of the SQT incorporating multivariate analysis of community structure, and laboratory-based biomarker assessment, has facilitated the establishment of a sound basis for understanding the nature of the chemical impact from the individual level to the overall ecology of the affected aquatic habitats of Deceitful Cove in the lower Tamar River

estuary. However, this work can only be considered as an extended preliminary study, and serves to emphasise the necessity for further investigation.

Clearly, several questions remain unanswered. A comprehensive field investigation is necessary to determine whether dredging would be the most effective and safest method of remediating Deceitful Cove. Dredging results in gross disturbance and re-suspension of buried contaminants. There is a need to assess the potential acute toxicity of the suspended and dissolved contaminant fraction of the dredged material remaining in the water column. Less aggressive alternatives might also be considered, such as natural or enhanced natural recovery. Once pollution prevention, source control, and re-suspension prevention measures are in place, allowing natural recovery assumes that contaminants would be lost to the biotic portion of the ecosystem through eventual burial (Phillips & Rainbow, 1993), preferably within a reasonable period of time (Chapman *et al.*, 1998). Enhanced natural recovery speeds up the remediation process by placing a layer of clean material over the contaminated sediment without disturbing the sediment or destroying the benthic community (Chapman *et al.*, 1998). Although both these remediation strategies are attractive in terms of containing and reducing metal contamination in Deceitful Cove, the wider issue of the biomagnification potential of persistent organic contaminants detected in sediments and benthic finfish in the Deceitful Cove - Port Dalrymple region will also need to be addressed.

The outcome of the project has been an improved understanding of factors affecting the quality of marine sediments and remediation of marine sediments. The multidisciplinary approach of this study has facilitated in the construction of the broader picture of the processes and potential causal links between the chemistry, ecology and toxicity elements of the shallow subtidal sediments in northern Tasmania. It has helped to clarify the potential routes of contaminant exposure, what contamination levels may cause toxicity and what effects the toxicity may have on the ecology of the region. The results have not only increased our knowledge of ecotoxicology of the investigated sediments, but also have practical applications for industry in the area of environmental management and recovery

of contaminated benthic habitats. The findings will improve interpretation of results of future chemical analysis and prediction of recovery of sediment quality over time.

Finally, the importance and value of these marine sediments must be stressed. Subtidal sediments are not only a fundamental ecological component contributing to the rich species and habitat biodiversity of the coastal zone, but from an economic and public interest perspective, they also offer refuge and feeding grounds for commercial and recreational fisheries species. Coastal estuarine sediments also provide valuable areas in which to study the extraordinarily complex relationships between the biological, chemical and toxicological components within the aquatic marine ecosystem, as demonstrated in this study.

General

References

Attril, M. J. & Depledge, M. H. (1997). Community and population indicators of ecosystem health - targeting links between levels of biological organisation. *Aquatic Toxicology, 38*(1-3), 183-197.

Burton, G. A. (1992). Plankton, Macrophyte, Fish, and Amphibian Toxicity Testing of Freshwater Sediments. In B. G. Burton (Ed.), *Sediment Toxicity Assessment* (pp. 167-182). London: Lewis Publishers.

Ciarelli, S. Vonck, W. A. P. M. A., Vanstraalen, N. M., & Stronkhorst, J. (1998). Ecotoxicology assessment of contaminated dredged material with the marine amphipod *Corophium volutator. Archives of Environmental Contamination and Toxicology, 34*(4), 350-356.

Chapman, P. M. (1990). The Sediment Quality Triad approach to determining pollution-induced degradation. *Science of the Total Environment, 97/98*, 815-825.

Chapman, P. M. (1995). Bioassay testing for Australian as part of water quality assessment programs. *Australian Journal of Ecology, 20*, 7-19.

Chapman, P. M., Wang, F., Janssen, C., G., P. & Allen, H. E. (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability , risk assessment, and remediation. *Canadian Journal of Fisheries & Aquatic Science, 55*, 2221-2243.

Courtney, W. A. M. (1980). Accumulation of polychlorinated biphenyls in turbot (*Scolphthalmus maximus*) from sea water sediments and food. *Helgolander Meeresunters, 33*(333-339).

Edgar, G. J., Barrett, N. S., Graddon, D. J. & Last, P. R. (1999). The conservation significance of estuaries: a classification of Tasmanian estuaries using ecological, physical and demographic attributes as a case study. *Biological Conservation (In press).*

Forstner, O. (1987). Sediment associated contamination - an overview of scientific basis for developing remedial options. *Hydrobiologia, 149*, 221-246.

Gawne, K. M. & Richardson, B. J. (1992). *An investigation into the distribution and accumulation of selected chemical species in the waters, sediments and biota of the lower Tamar estuary, Tasmania* : Deakin University Centre for Biological and Chemical Research.

Gray, J. S., Clarke, K. R., Warwick, R. M. & Hobbs, G. (1990). Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. *Marine Ecology Progress Series, 66*, 285-299.

Ingersoll, C. G. (1995). Sediment tests. In G. M. Rand (Ed.), *Fundamentals of Aquatic Toxicology* (pp. 231-256). London: Taylor & Francis.

Ingersoll, C. G., Ankley, G. T., Baudo, R., Burton, G. A., Lick, W., Luoma, S. N., MacDonald, D. D., Reynoldson, T. B., Solomon, K. R., Swartz, R. C. & Warren-Hicks, W. J. (1997). Workgroup summary report in uncertainty evaluation of measurement endpoints used in sediment ecological risk assessments. In C. G. Ingersoll, T. Dillon & G. R. Biddinger (Eds.), *Ecological risk assessment of contaminated sediments.* (pp. 297-352). Pensacola: SETAC Press.

Klerks, P. L. & Weis, J. S. (1987). Genetic adaptation to heavy metals in aquatic organisms: A review. *Environmental Pollution, 45*, 173-190.

Long, E. R. & Chapman, P. M. (1985). A Sediment Quality Triad: Measures of sediment contamination, toxicity and infaunal community composition in Puget Sound. *Marine Pollution Bulletin, 16*(10), 405-415.

Luoma, S. N. (1990). Processes affecting metal concentrations in estuarine and coastal marine sediments. In R. W. Furness & P. S. Rainbow (Eds.), *Heavy metals in the marine environment* (pp. 51-56). Boca Raton: CRC Press.

Luoma, S. N. (1996). The developing framework if marine ecotoxicology: Pollutants as a variable in marine ecosystems? *Journal of Experimental Marine Biology and Ecology, 200*, 29-55.

Matthiessen, P., Bifield, S., Jarrett, F., Kirby, M. F., Law, R. J., McMinn, W. R., Sheahan, D. A., Thain, J. E. & Whale, G. F. (1998). An assessment of sediment toxicity in the River Tyne estuary, UK by means of bioassays. *Marine Environmental Research, 45*(1), 1-15.

Matthiessen, P., Blackburn, M., Kirby, M., Law, R., Sheahan, D. & Thain, J. (1995). Toxicity of estuarine waters and sediments resulting from industrial chemicals. *Marine Environmental Management Review of 1994 and Future Trends, 2*(4), 23-26.

Maund, S., Chapman, P., Kedwards, T., Tattersfield, L., Matthiessen, P., Warwick, R. & Smith, E. (1999). Application of multivariate statistics to ecotoxicological field studies. *Environmental Toxicology and Chemistry, 18*(2), 111-112.

Miedecke, J. (1992a). *Deceitful Cove rehabilitation study. Final Report.* : Meidecke and Partners.

Miedecke, J. G. (1992b). *Deceitful Cove Bioaccumulation Investigation* . Hobart: John Miedecke & Partners.

Mondon, J. A. (1996). *Deceitful Cove Ecotoxicology Preliminary Survey for Comalco and TEMCO* . Launceston: UniTas Conultancy.

Mondon, J. A., Nowak, B. F. & Sodergren, A. (1999b). Persistent organic pollutants in oysters *Crassostrea gigas* and sand flathead *Platycephalus bassensis* from Tasmanian estuarine and coastal waters. *Marine Pollution Bulletin (submitted).*

Peddicord, R., Chase, T., Dillon, T., Mc Grath, J., Munns, W., van de Gucthe, K & van der Schalie, W. (1995). Navigational dredging. In C. G. Ingersoll (Ed.), *Ecological Risk Assessment of Contaminated Sediments* (pp. 41-72). Pensacola, Florida: SETAC Press.

Peters, L. D., O'Hara, S. C. M. & Livingston, D. R. (1996). Benzo(*a*)pyrene metabolism and xenobiotic-stimulated reacyive oxygen species generation by subcellular fraction of larvae of turbot (*Scophthalmus maximus* L.). *Comparative Biochemistry & Physiology, 114C*(3), 221-227.

Phillips, D. J. H. (1980). *Quantitative aquatic biological indicators: their use to monitor trace metal and organochlorine pollution.* London: Applied Science Publishers.

Phillips, D. J. H. (1993). Bioaccumulation. In P. Calow (Ed.), *Handbook of Ecotoxicology* (Vol. 2, pp. 378-396). London: Blackwell Scientific Press.

Phillips, D. J. H. & Rainbow, P. S. (1993). *Biomonitoring of trace aquatic contaminants.* London: Elsevier Science Publishers.

Seelye, J. G., Hesselberg, R. J. & Mac, M. J. (1982). Accumulation by fish of contamination released from dredged sediments. *Environmental Science & Technology, 16*, 459-463.

Silby, R.M & Calow, P. (1989). A life cycle theory of response to stress. *Biological Journal of Linnean Society*, 37, 101-116

Simkiss, K. & Taylor, M. G. (1989). Metal fluxes across the membranes of aquatic organisms. *Review of Aquatic Science, 1*, 173-188.

Stegeman, J. J. & Lech, J. J. (1991). Cytochrome p-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environmental Health Perspectives, 90*, 101-109.

Tagaza, E. (1995). Understanding the coastal zone. *Ecos, 83*, 10-11.

Underwood, A. J. (1996). Detection, interpretation, prediction and management of environmental disturbances: some roles for experimental marine ecology. *Journal of Experimental Marine Biology and Ecology, 200*, 1-27.

Waldichuk, M. (1985). Biological availability of metals to marine organisms. *Marine Pollution Bulletin, 16*, 7-11.

Warwick, R. M. (1993). Environmental impact studies on marine communities: Pragmatical considerations. *Australian Journal of Ecology, 18*, 63-80.

GLOSSARY

GLOSSARY

This glossary defines key terms that occur in the text.

a priori	Prior to the event.
absorption	A process in which a solute becomes physically associated with a porous sorbent.
acute test	A test performed in the short term (relative to generation time) and usually but not always at high concentrations.
additive	Refers to mixtures of toxicants; toxic effect in combination = sum of separate parts.
anaerobic	In the absence of oxygen.
adsorption	A process in which a solute becomes physically associated with a solid sorbent.
anthropogenic	Produced as a result of human activity.
baseline study	Data collected to define the present state of environmental (eg. metal concentrations) or biological (eg. assemblage) components, against which future observations can be assessed.
benthic	Living on, or close to, the floor of the sea, or other water body. Also known as **demersal.**
bioaccumulation	The net accumulation of a substance by an organism as a result of uptake from all environmental sources.
bioassay	An experiment in which single test-species are exposed in the laboratory to samples of a field sediment (or extracts of this) potentially containing one or more contaminants, with the aim of measuring possible biological effects of those contaminants.
bioavailable	Describes a chemical present in a form or phase which causes biological responses in one or more of the species in which it comes into contact.
bioconcentration	The net assimilation of a substance by an aquatic organism as a result of uptake directly from aqueous solution.
biodegradation	A microbiologically mediated process that chemically alters the structure of a chemical, the result being the break-up of the chemical.
biological response	Refers to the effect elicited by contamininants at any biological level.
biomagnification	Food chain effect; substances bioaccumulated and bioconcentrated at one trophic level are concentrated at higher trophic levels.
biomarker	Any morphological, physiological or behavioural changes that are taken as indicators of pollution / stress. May be generic or specific.
biotransformation	A microbiologically mediated process that chemically alters the structure of a chemical, but does not necessarily degrade it.
bioturbation	Mixing of sediments by biological action eg. burrowing.
carcinogenic	Chemicals, ionizing radiation, and viruses that cause or promote the development of cancer.

chronic test	A test in which an organism is observed over along period of time which may include a substantial portion of its life cycle.
clade	All of the hierarchical branching tree beyond some branching point.
community	An assemblage of populations of different species within a specified location in space and time.
complexation	A process in which two or more solutes join chemically to form a new chemical complex.
cumulative effects	The combined effects of multiple stressors (eg. metals and organics) over time.
depuration	Loss of a substance from an organism (eg. metabolic breakdown) or passive process when the organism is placed in an uncontaminated environment.
direct effect	The stressor acts on the ecological component itself, rather than through effects on other components of the ecosystem.
diversity	This is usually expressed as species richness (i.e. number of species) and evenness (i.e. proportional representation of each species).
EC_{50}	Concentration (dose) that effects designated criterion (eg. growth) of 50% population observed. Also known as median effect concentration / dose.
ecological component	Any part of an ecological system, including individuals, populations, communities, and the ecosystem itself.
ecological risk assessment	The process that evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors.
ecosystem	The biotic community and abiotic environment within a specified location in time and space.
ecotoxicology	The study of pollutants in ecosystems.
estuary	A coastal body of water which has a free conection with the open sea and where fresh water is mixed with sea water.
euryhaline	Species able to tolerate a wide variation of osmotic pressure (salinity).
exposure	Co-occurrence of, or contact between, a stressor and an ecological component.
fitness	Used in a Darwinian sense referring to capacity to reproduce and survive.
habitat	The physical condition in which a species is found (e.g. shallow subtidal sediment).
hydrophilic	Molecules that are water attracting or attracted to water.
hydrophobic	Applied to molecues that resist wetting or solvation by water (eg. poalr molecules).
immune	Innate or acquired resistence to disease.
impact	A perturbation causing an alteration in a measured variable of a population (e.g. abundance) or assemblage (eg. diversity).
in situ	In the original location.
induction	"Switching on" of translation and transcription processes leading to synthesis and activation of a particular enzyme.

infauna	Lives in sediment of aquatic systems.
lipophilic	Fat soluble.
macrofauna	Organisms associated with sediment and retained in a geological sieve of 1 mm.
Microtox®	A test involving the "luminous" marine bacterium *Vibrio fischeri*. Changes in light output are measured as indicators of stress.
monitoring	Sampling in time with adequate replication to detect variation over a temporal range from short to long time periods.
multivariate analysis	The analysis of multiple variables (eg. species, environmental variables), which may have been measured at multiple levels (i.e. factors; e.g. times, locations, sites, habitats), allowing simultaneous analysis of multiple dependent and multiple independent variables).
necrosis	Dead or dying cells or tissues within the living body.
non-parametric statistics	Distribution-free methods in which data are analysed according to rank.
organic compounds	Any chemical based on carbon as the main structural element, including non-natural or manufactured organic chemicals (eg. PCBs, PCDDs, PCDFs).
overlying water	The water in the test chamber over the sediment in a bioassay.
pollution	The introduction by humans, directly or indirectly, of substances or energy into the aquatic environment resulting in deleterious effects.
population	An aggregate of individuals of a species within a specified location in time and space.
porewater	Water occupying space between sediment particles. Also known as **interstitial water**.
phagocyte	A cell capable of ingesting bacteria, foreign particles and other cells.
press	Refers to a type of impact that is sustained.
pulse	Refers to a type of impact that is not sustained.
recovery	Partial or full return of a population or community to a condition that existed before the introduction of the stressor.
redox potential	Measure of the proportion of oxidized to reduced substances.
reference sediment	An uncontaminated sediment whole sediment , representing the test (contaminated) sediment in all possible characteristics.
remediation	Clean up of contaminated sites.
risk assessment	A process by which measures are taken in the field to remove or reduce or minimise the expected environmental risks associated with the use of a substance or mixture of substances.
sediment	Particulate material which usually lies below water, having settled from the water column.
sedimentation	A process in which suspended solids, and chemicals, in a surface water are deposited in the bottom of the water body.
speciation	A term referring to the existence or formation of a variety of chemical complexes generally related to a central element.

spiked sediment	A sediment to which a material has been added for experimental purposes.
statistical inference	Where a null hypothesis is rejected at a predetermined level of probability (e.g. $P<0.05$, i.e. there is less than a 5% chance that the null hypothesis is correct).
stressor	Any physical, chemical, or biological entity which can induce an adverse response.
subtidal	Waters below the low-tide mark.
succession	A sequence of ecological events that relate to the colonisation and persistence of species in an assemblage through time.
synapomorphy	A shared derived characteristic.
synergistic	Refers to mixtures of toxicants, where the toxic effect in combination is greater than the sum of the effect of each toxicant in isolation.
systemic response	Effects that require absorption and distribution of the chemical to a site distant from the original contact or entry site.
toxicant	A chemical that has an adverse effect on organism(s).
toxicity	A measure of the harm that a substance can cause to biological systems.
toxin	A natural poison.
trace metals	For the purposes of this study includes heavy metals with atomic numbers between 21(scandium) and 92 (uranium), plus aluminium.
trophic levels	A functional classification of taxa within a community which is based on feeding relationships.
univariate analysis	Statistical methods used to analyse one dependent variable (e.g. number per unit area of an organism).
variables	The measurements taken at each level of sampling (e.g. species, measures of the environment).
whole sediment	Sediment and its associated porewater. Synonymous with bulk sediment.
xenobiotic	A chemical or other stressor which does not occur naturally in the environment.

REFERENCES

Calow, P. (ed.) (1993), Handbook of Ecotxicology. Vol.1, Blackwell Scientific Publications, London.

Ingersoll, C.G., Dillon, T. & Biddinger, G.R. (eds.) (1997), Ecological Risk Assessment of Contaminated Sediments. SETAC Press, Pensacola.

Kingsford, M. & Battershill, C. (eds.) Studying Temperate Marine Environments, Canterbury University Press, Canterbury.

Rand, G.M. (ed.) (1995), Fundamental of Aquatic Toxicology. Effects, Environmental Fate and Risk Assessment. Taylor and Francis, Washington.

APPENDICES

DECEITFUL COVE
PRELIMINARY SUBTIDAL BENTHIC SURVEY
(December 1995 / January 1996)

SECTION ONE
•
GENERAL INTRODUCTION

Marine sediments collect urban and industrial derived contaminants. Sediments are known to serve as both a sink and source for toxic contaminants through processes of deposition, diffusion, adsorption, re-suspension and emigration (Forstner, 1987). Continuous contaminant exchange between the water column and bottom sediments has the potential to affect the marine biota (Seelye *et al.*, 1982; Phillips, 1993). Uptake of dissolved heavy metals in the pore water, and synthetic compounds may be acutely toxic to some organisms and may also bioaccumulate, effectively dispersing the impact range of the contaminant away from the disposal site (Luoma, 1990).

Deceitful Cove in the Tamar estuary is perceived to be heavily contaminated by past industrial effluent. Discharge of wastes primarily from the Comalco and TEMCO industrial plants have concentrated heavy metal toxicants within the marine sediments of the cove (Miedecke, 1992). Suspicion of a relationship between chemical contamination and deleterious biological effects on the marine biota has prompted a remediation proposal to remove these waste sediments from Deceitful Cove. This precautionary action is premised on contaminated sediment posing a greater risk to the marine environment if left undisturbed than if removed.

The relationship between chemical contamination of marine sediments, pore waters and the marine biota of Deceitful Cove has not been established. Previous investigations of sediment quality within the region involved short term independent chemical analyses of

sediment, pore and surface waters, and animal tissues (Gawne & Richardson, 1992); (Miedecke, 1992). Bio-availability of chemicals is difficult to measure chemically (Chapman, 1990), and the interpretation of biological significance is unclear from these studies. Understanding the factors affecting quality and redemption of marine sediments requires a multi-disciplinary study comprising simultaneous investigation of chemical contamination, toxicity and ecology of sediments. The multi-disciplinary approach facilitates a close approximation of what contamination levels might cause toxicity and what effects the toxicity will have on the ecology of the area.

Research aims and methodology

The aims of this preliminary survey were twofold:

- to determine that the geochemistry, toxicity and ecology of subtidal sediments of Deceitful Cove is similar to that of other estuarine locations over the short term.
- to generate baseline data from which to establish over the long term if Deceitful Cove sediment quality is affected by season or year of sampling, and if it presents a toxic hazard to the surrounding ecosystem.

The relationship between and variation within toxicity, chemical composition and animal communities were investigated through a field program. Samples were collected at four locations: Deceitful Cove, East Arm, Squeaking Point and North East Arm. All locations were sampled twice during the summer period and on each occasion samples were collected from four replicate sites. Sampling was conducted at the lowest tide and involved collection of sediments from approximately 0.5 m – 1 m depth below the water surface. Selection of intermediately polluted sites at East Arm and effectively unpolluted sites at Squeaking Point and North East Arm were based on the assumption that geochemistry of sediments were relatively similar for all locations. Figure1 illustrates the geographical position of sites at Deceitful Cove, East Arm, Squeaking Point and North East Arm. The position of sites within each location were marked using a series of visual fixes from the

shore. To increase accuracy in pinpointing sites from the water an Eagle AccuNav Sport[a] GPS (Global Positioning System) was trialed during the latter half of the field survey.

Toxicity of pore water and subtidal sediments were assessed using routine bioassays chosen on the basis of their sensitivity to a wide range of organic and inorganic pollutants, and routine availability. Standard and Solid-Phase Microtox® tests together with an algal growth inhibition test, formed the first tier ecotoxicology assessment.

The sensitivity of algae and reproducibility of the *Nitzchia closterium* growth inhibition toxicity test offers convenient and cost-effective tools for monitoring potential toxicity of environmental samples, complex effluents and specific chemicals (Stauber, 1995). Any adverse impact on algae may affect, directly and indirectly, organisms at higher trophic levels. Phytoplankton exhibit short life cycles, consequently alterations in algal density indicates a rapid response to environmental change (Lewis, 1995). A local isolate of the marine diatom *Nitzchia closterium* was chosen for the algal bioassay due to its wide spread distribution in the Australian temperate coastal zone. Exponentially growing *N.closterium* are exposed for 72 hours to pore water extracted from sediments. The pore water is considered toxic when a statistically significant, dose-dependent inhibition of algal biomass occurs.

Microtox® assays measure the effects of contaminants on light production of luminescent bacteria *Vibrio ficsheri* (Ankley *et al.*, 1989; Ringwood *et al.*, 1997). Light is emitted as a result of a metabolic pathway that is intrinsically linked to cellular respiration, so that disruption of normal cellular metabolism causes a decrease in light production (Ringwood *et al.* 1997). The suite of Microtox® tests, Solid Phase and Standard, were chosen to measure the total effective toxicity of the solid as well as liquid component of whole marine sediment, detecting soluble as well as insoluble organic and inorganic toxicants.

Estimation of the species abundance and richness of sediment macroinvertebrate fauna was determined from surface sediment core samples. Sediment and pore waters were analysed for trace metals, polycyclic aromatic hydrocarbons (PAH), total petroleum hydrocarbons

(TPH) and benzene, toluene, ethyl benzene and xylene (BTEX). Granulometry of each location was undertaken to determine the sediment type and pore water volume.

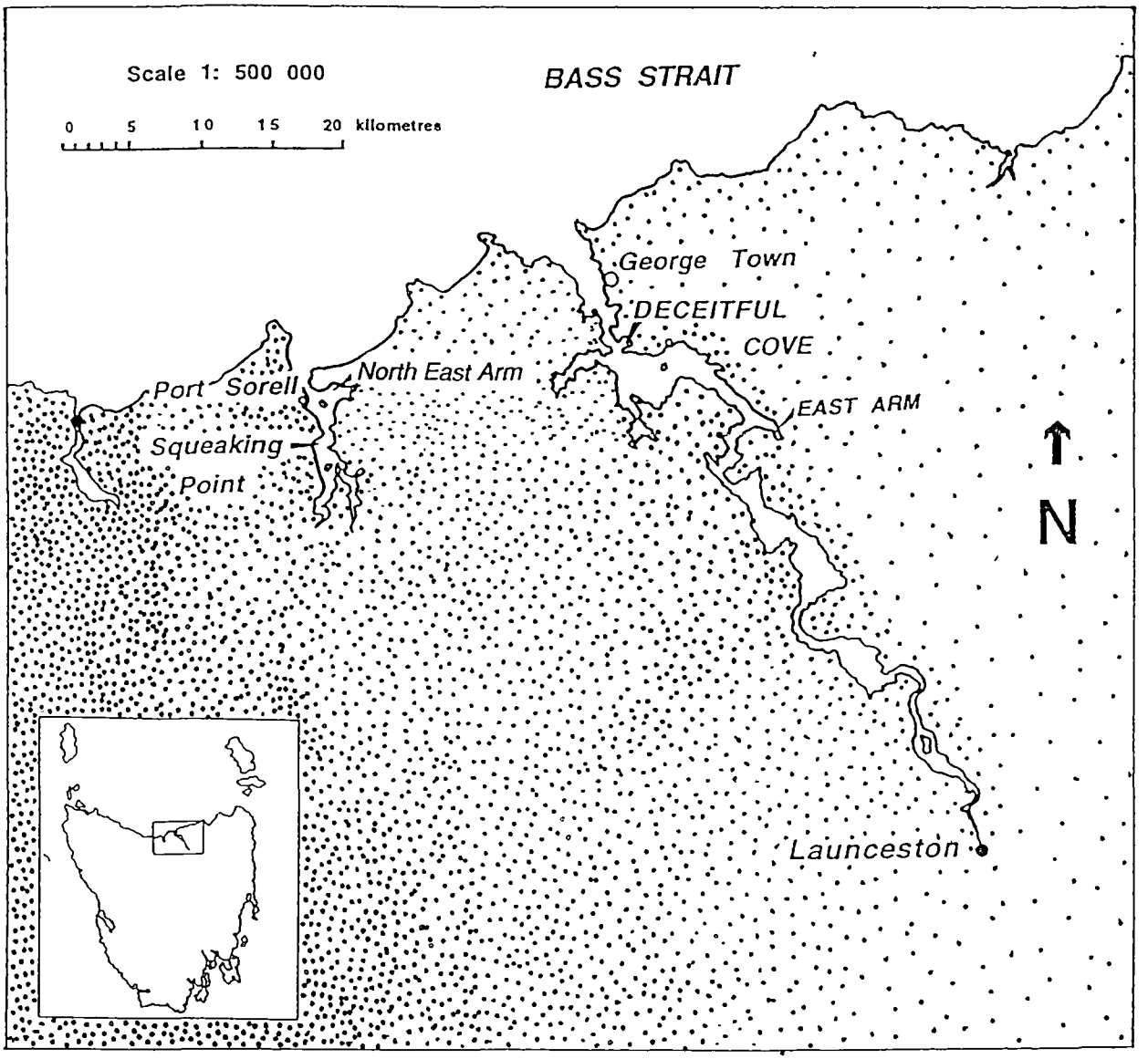


Figure 1. Position of sampling sites on the Tamar River and Port Sorell estuaries.

SECTION TWO

•

BIOASSAYS

Sediment collection and pore water extraction

Vacuum-operated pore-water extractors were used to collect pore water for bioassay tests. A fused-glass air stone attached with a section of Teflon® tube to a 50mL acid washed polypropylene syringe (Figure 2.1) was inserted into extracted pooled sediment cores. A vacuum created by retracting and bracing the syringe plunger drew water through the air-stone and up into the syringe. Sediment surrounding and accumulating on the surface of the air stone acts as a filter resulting in the collection of clear pore water, thus eliminating the need for filtration or centrifugation prior to toxicity testing (Winger & Lasier, 1991). The potential for small amounts of contaminants to be adsorbed to the extraction device is considered to be non-significant in either detection levels, or reducing or altering toxicity of pore water considering the inherent variability of field samples (Winger & Lasier, 1991).

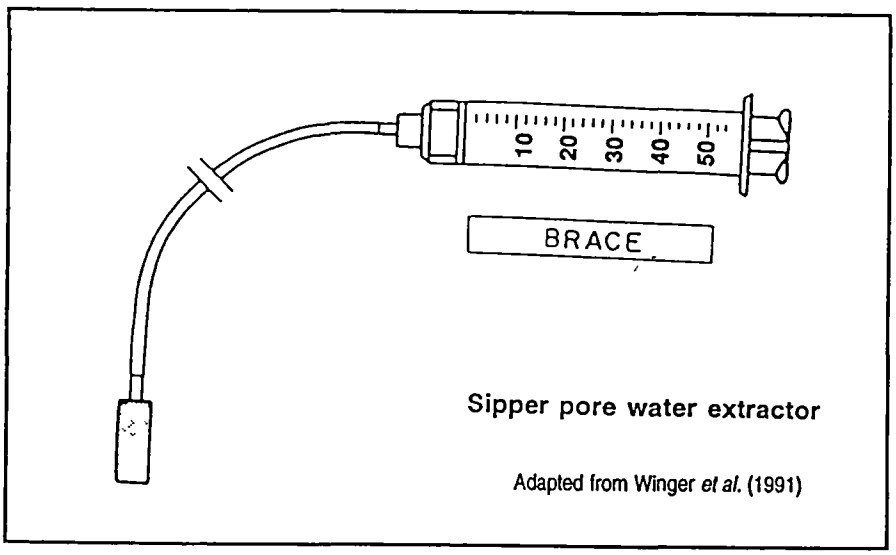


Figure 2.1. The sipper device used for extraction of pore water from subtidal sediments.

Extraction of pore water did not take place under in-situ conditions. Initial trials to perfect *in-situ* collection highlighted two inhibiting factors. Firstly, the time required to collect sufficient volumes of water from a constantly moving dinghy necessitated simultaneous deployment of several sippers. Extraction time increased significantly with finer sediments. Windy conditions and a non-stationary platform meant that it was extremely difficult to keep sippers embedded in one place over any extended period of time. Secondly, any movement of the Teflon® tubing attached to the fused-glass stone disturbed the surface sediment; a problem exacerbated during "less than dead calm" conditions. *In-situ* extraction could therefore represent unknown samples as surface water would conceivably mix with interstitial water during the extraction process. Therefore, the sediment from which pore water has been collected would not be fixed in terms of volume or source of replacement water.

Whole sediment surface core samples (0-10 cm depth) were placed in sealed acid-washed containers. Surface water transferred during to the process was carefully removed from the sediment by pipette. Pore water was extracted from the sediment in the laboratory within a few hours of collection due to unpredictable changes in pore water toxicity that have been observed during sediment storage (Carr &Chapman., 1995).

Pore water was stored at -20°C immediately after extraction. The difference in water quality parameters between fresh and frozen/thawed samples is generally not considered to be significant (Carr & Chapman, 1995). Assessment of the effects of storage temperature on Deceitful Cove pore water indicated samples are best stored at -20°C to limit the potential loss of toxicity when stored at +4°C (Appendix 2). Sediment cores were collected using 70 mm benthic core tubes which were inserted by hand into sediment to a depth of 10cm below the sediment-water interface. Corers were employed to minimise loss of surface sediment due to bow wave generation by benthic grabs and similar devices, and to minimise potential long-term gross disturbance of the sediment. Whole sediment cores were placed in inert acid-washed containers and kept in an ice-cooled cooler during transportation to a +4°C refrigeration unit. Sediment samples were stored up to 4 days during which time Solid Phase Microtox® bioassays were conducted. The duration of

storage falls within the recommended maximum storage period of between 5 to 7 days at +4°C, thus minimising a potential source of systematic bias (Anderson *et al.* , 1987; Swartz, 1987; Mondon, unpublished (Appendix 2)).

Introduction

The bioavailability of chemicals in sediments is best determined by means of bioassays. Bioassays allow direct assessment of whether a biological response to a mixture of chemicals in a sediment can occur. Ranking of relative toxicity is also possible and is of particular importance when considering remediation proposals for particular sites.

Uptake of toxicants by marine organisms is controlled to a certain degree by the concentration of toxic substances dissolved in porewaters (for review see (Chapman *et al.*, 1998)). However, as pore water is not the sole conduit for uptake, two of the three bioassay tests involved exposure of marine micro-organisms to pore water, the third involved exposure to sediment. By removing pore waters from sediments, effects of natural sediment factors such as grain size are eliminated before testing and the rate of change in redox conditions and metabolite concentrations is suppressed (Chapman, 1989). Unfortunately if anoxic pore waters are exposed to oxygen during extraction from the sediment, or during the bioassay procedure, precipitation of Fe and Mn oxides occurs immediately (Troup *et al.*, 1974). As a consequence trace elements co-precipitating with the oxides are removed from solution. The Standard Microtox® and algal growth inhibition tests used in this survey may therefore under-estimate metal toxicity due to this factor.

Methods

Algal growth inhibition test

All glassware was washed in non-phosphate detergent, rinsed with de-ionised water, soaked in 10% HNO_3 overnight and air dried prior to commencement of the test.

The algal inoculum was prepared from a 5 day old stock culture of *Nitzchia closterium*. The culture was centrifuged at 2500 rpm for 7 minutes and the supernatant removed with a pasteur pipette. The culture was then washed 3 times by re-suspending the pellet in 25 mL 0.2μm filtered seawater, "vortexing" the suspension with a pasteur pipette and centrifuging for 7 minutes. The pellet was then re-suspended in 15 mL 0.2 μm filtered seawater.

A duplicate set of a control, plus five 2:1 serial dilutions of pore water (100%, 50%, 25%, 12.5% and 6.25%) made up to 50 mL using 0.2 μm filtered seawater, were placed in 200 mL Erlenmeyer flasks. 0.5 mL 26mM sodium nitrate plus 0.5 mL 1.3mM potassium dihydrogen phosphate were added to the controls and pore water dilutions. Algal inoculum was then added to the flasks resulting in a final culture density of $4\text{-}6*10^4$ cells mL^{-1}. The test cultures were incubated over a daylight florescent light box for 3 days: 12 hours light / 12 hours dark. Cell density was determined using an Improved Neubauer haemocytometer.

Microtox® MDSP test

Initial range finding trials using a dilution sequence to assess the acute sublethal toxicity of contaminants in terms of an EC_{50} was unsuccessful. An alternative assay, the Microtox® Medical Device Screening Protocol (MDSP) (Microbics Corporation), was used to assess non-relative toxicity of replicated pore water samples (Microbics Microtox® Manual). Luminescent bacteria, *Vibrio fischeri,* were exposed to non-diluted pore water samples in a replicate four-tube plus control series. The test endpoint, light emission inhibition, was determined at 15°C over 30 minutes, using a Microtox® M500 Toxicity Analyser and expressed as percentage light emission inhibition at neat (49.5%) pore water concentration. The higher the light emission inhibition value, the higher the toxicity of the sample.

Solid Phase Microtox® test

The procedure used was supplied by the CSIRO (Commonwealth Scientific and Industrial Research Organisation) and assesses the acute sublethal toxicity of contaminants in terms of an EC_{50}.

Ten cuvettes labelled A1 to A5 and B1 to B5 were placed in the M500 Toxicicty Analyser incubator wells. 1000 µL reconstitution solution was then added to the cuvette in the reagent well and allowed to stand for 5 minutes. Ten Microtox® solid phase tubes, labelled A1 to A5 and B1 to B5, were placed in a test tube rack. 400 mg of sediment which had been mixed thoroughly, centrifuged to separate pore water for removal, and then mixed again, was placed in solid phase tube B5. Microtox® reagent was removed from the freezer and reconstituted by dumping the reconstitution solution into the reagent vial. The mixture was swirled two times and poured back into the reagent cuvette, which was then returned to the reagent well before being mixed by micropipettor 20 times. The reconstituted reagent was then added to a bottle of Microtox® Solid Phase diluent (Microbics Corporation) and mixed by inverting the bottle 5 times. 2000 µL solid phase diluent-reagent was placed into the test tubes labelled A1 to A5 and B1 to B4. A timer was set for 20 minutes, during which time 4000 µL solid phase diluent-reagent mixture was added to the sediment in test tube B5, covered with Parafilm® and mixed by shaking, suspending as much of the sample as possible. Two-fold serial dilutions were made by transferring 2000 µL from tube B5 to B4, B4 to B3 and so on. At each transfer the contents of the receiving tube was mixed 20 times by micropipettor before transfer to the following tube. 2000 µL from tube A2 was discarded (0% pore water concentration). When the timer signalled the end of the 20 minute period, Microtox® filter columns were inserted into each tube (starting with A1) and gently pushed down to filter out the coarse particles. 500 µL filtrate from each filter column was transferred to its corresponding cuvette in the M500 Toxicicty Analyser and allowed to sit for 5 minutes, after which time the light emission levels were read.

Results and discussion

Algal growth inhibition test

Sampling time proved to be a non-significant factor which enabled pooling of data. Growth inhibition of *N.closterium* differed significantly between locations ($\chi^2 15 = 175$, $P<0.001$). Deceitful Cove exhibited an inhibition in growth when exposed to 100% pore water, compared to East Arm, Squeaking Point and North East Arm; each of which displayed a trend towards enhanced rather than reduced growth (Figure 2.2). Squeaking Point revealed the highest phytoplankton growth rate of the latter three locations. Deceitful Cove exhibited the lowest EC_{50} value (Figure 2.3). East Arm, Squeaking Point and North East Arm did not exhibited an EC50 value.

The *N.closterium* results indicate that in general, lower levels of trace metals in pore water, relative to Deceitful Cove, stimulate algal growth. The presence of small concentrations of Mn, and to a lesser extent Fe, in marine waters is known to alleviate copper toxicity to *N.closterium* (Stauber & Florence, 1985). Considering the high levels of Mn at Deceitful Cove and comparatively low levels of Cu relative to other locations, the inhibited growth rate at Deceitful Cove points to toxicity from contaminant sources other than Cu at this site. It is possible that the presence of fine particulate material, noted at the bottom of the pore water samples, may be masking the toxicity of sipper collected pore water samples, due to the strong binding affinity of metals to particulate organic matter (Chapman *et al.*, 1998). If this is the case, the toxicity of Deceitful Cove pore water would appear to be less toxic than it really is, by the decrease in bioavailability of metals in solution. To minimise the potential error associated with particulate material not filtered out during sipper extraction, it is advisable to centrifuge pore water before its use in bioassays (R.S. Carr, *pers. comm.*).

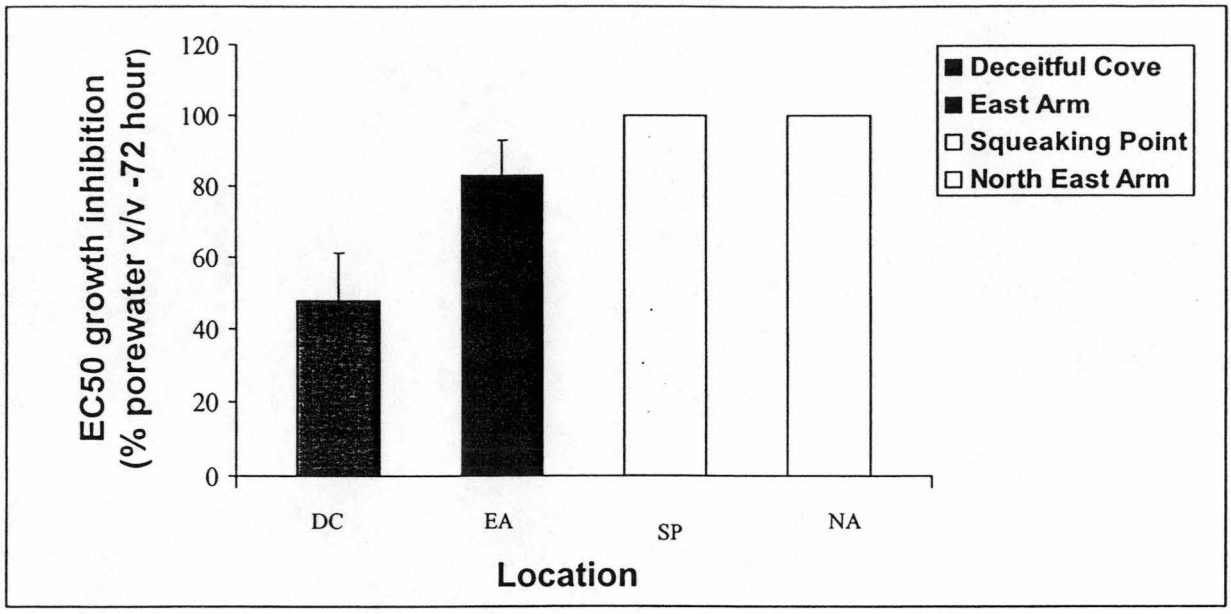


Figure 2.2. ***Nitzchia closterium*** **growth inhibition with exposure to neat pore water.**

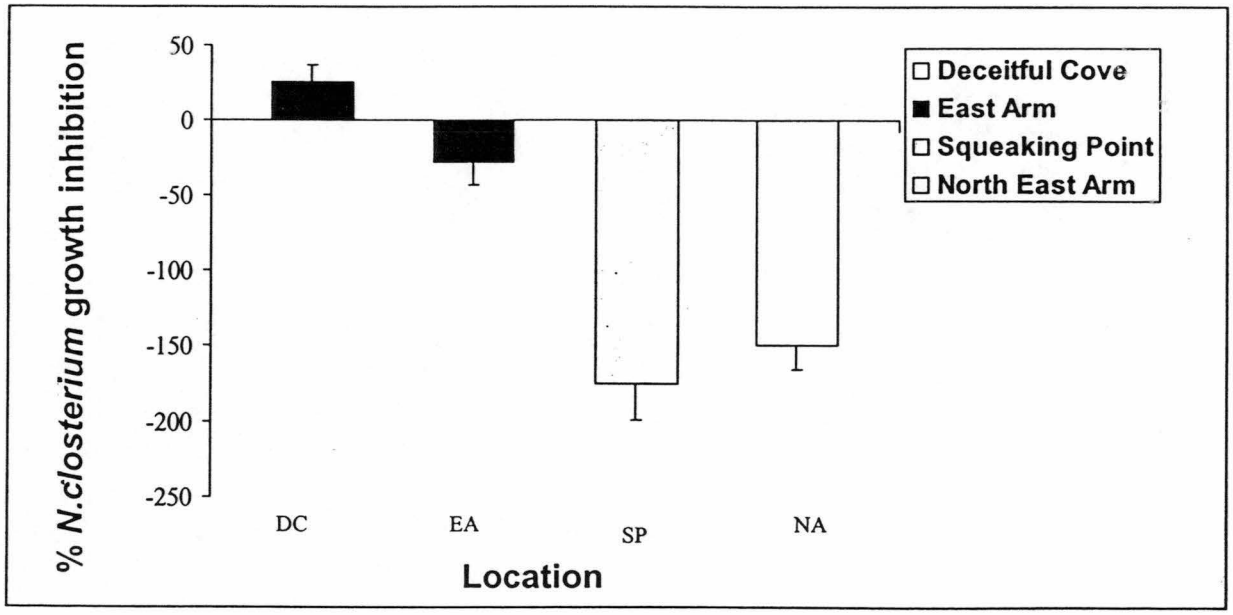


Figure 2.3. ***Nitzchia closterium*** **EC_{50} growth inhibition.**

The variability amongst replicates in the assays may be attributed to two factors. It is feasible that changes in trace metal chemistry with respect to oxidation of pore water samples may have suppressed toxicity in some assays and not others. Secondly, suspected contamination of the *N.closterium* stock culture may have been influential in generating the degree of variability amongst samples.

Microtox® MDSP test

Sampling time was non-significant allowing pooling of data to take place. *Vibrio fisheri* light reduction emission levels differed significantly between Tamar River locations and those at Port Sorell (χ^2 7= 28.12, P<0.001). Deceitful Cove and East Arm exhibited higher rates of light emission reduction overall compared to Squeaking Point and North East Arm (Figure 2.4).

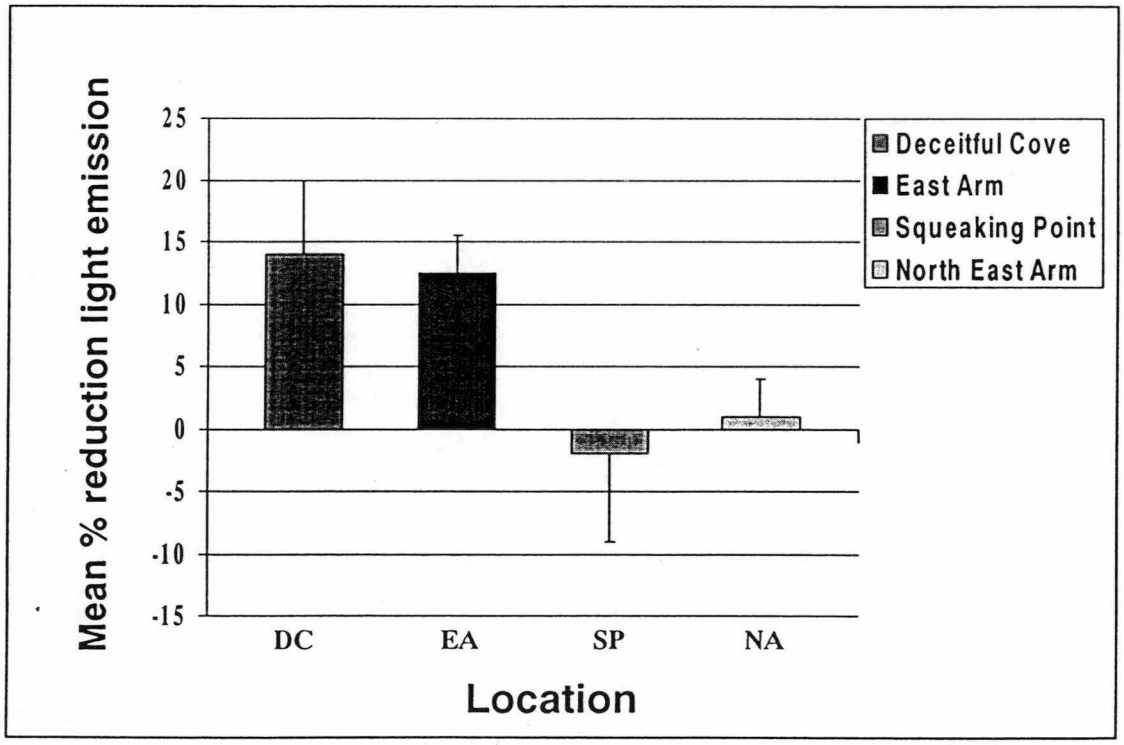


Figure 2.4. Microtox® MDSP reduction in light emission with exposure to pore water.

Whilst the Microtox® MDSP assays indicate higher levels of toxicity at particular sites, the test appears less sensitive to complex contaminants than expected. Stimulation effects on light illumination observed during the first fifteen minutes of exposure to some pore water samples correlates with observations recorded from Microtox® bioassay tests conducted on leachate extracted from steelworks effluent (Skacel *et al.*, 1993). It is possible that short-term bacterial adaptation during the early phase of the test results in comparatively higher endpoint values, particularly when dealing with predominantly metal based contaminants rather than organic compounds.

Solid Phase Microtox® test

The difference in sampling time was non-significant, thereby allowing pooling of data. Microtox® EC_{50} values differed significantly between survey locations (χ^2 3= 18, $P<0.001$), with East Arm exhibiting the highest toxicity with the lowest EC_{50} value (Figure 2.5). Despite DC indicating a comparatively lower toxicity level relative to other locations, sediments from all sites are considered toxic with mean EC_{50} values occurring within a narrow exposure range of 0.5% -1.35% wt. of sediment per volume diluent.

The Solid Phase test exhibits the greatest sensitivity to complex contaminants with test organisms coming into direct contact with toxicants bound to particulate matter. The total effective toxicity of the solid material exceeds that of pore water and is supported by evidence of markedly higher concentrations of trace metals in sediment compared to pore water. Whereas pore water bioassays are far less likely to detect insoluble toxicants, they do indicate relative toxicity to soluble contaminants.

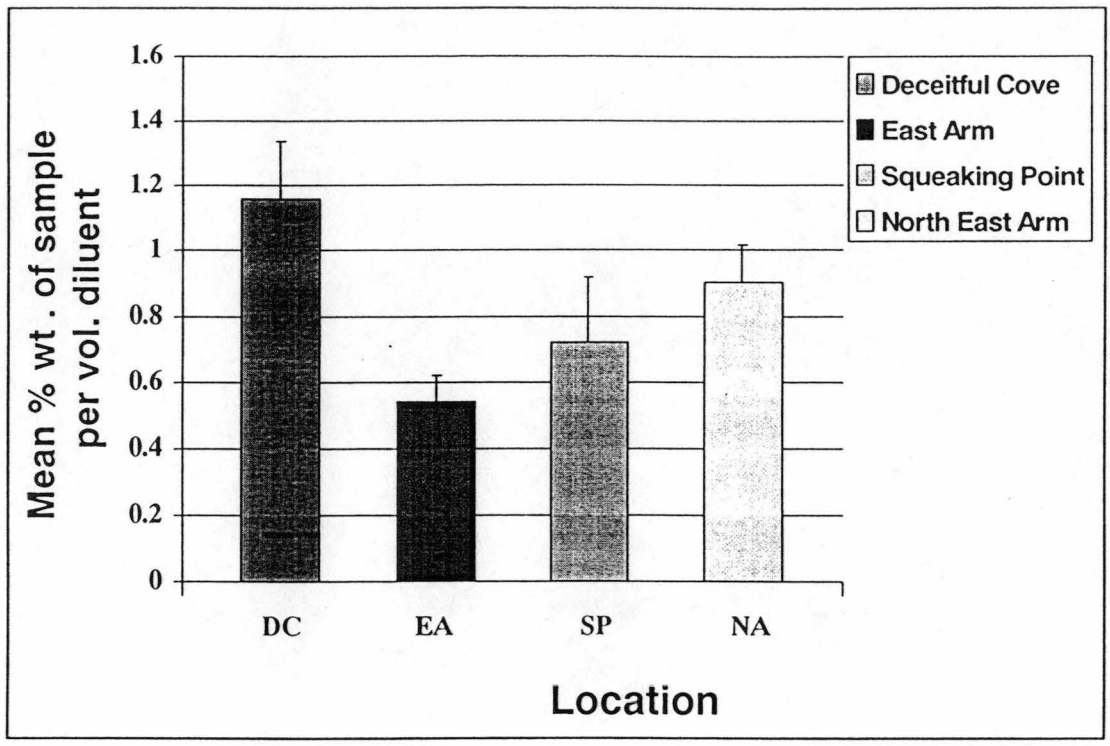


Figure 2.5. Microtox® Solid Phase EC_{50} reduction in light emission.

Both pore waters and sediment, aerated during toxicity tests, estimated the potential toxicity of uncovered or re-suspended sediments rather than simulating in-situ conditions. The precipitation of toxicants when pore water is oxidised, is of importance as a potential source of false-negative responses in bioassays, but not necessarily deleterious when anoxic pore waters are exposed to surface waters. Reduced pore waters with elevated concentrations of reduced Fe / Mn in solution may not impact greatly on the adjacent ecosystem if exposure to oxygen through a sediment disturbance such bioturbation or storm activity immobilises the potential toxicity of that pore water via the co- precipitation of metals.

The elevated levels of solid phase toxicity recorded in samples from the Port Sorell reference locations (Squeaking Point and North East Arm), may be due to a decrease in light illumination caused by adhesion of the bacteria to the sediment. Silty sediments are known to attract *Vibrio fischeri*, so that fewer bacteria present in the filtrate, will result in a lower light reading.(Ringwood *et al.*, 1997). Although, Deceitful Cove sediment contains the highest silt content, it exhibits the highest light emission. Conversely, unmeasured contaminants may be present in Port Sorell sediments which are toxic to *Vibrio fischeri*. The inconclusive nature of solid phase results suggests further work is necessary to determine the reason accounting for the apparent toxicity of the reference samples.

SECTION THREE

•

CHEMICAL ANALYSIS

Introduction

Contaminants entering aquatic systems become associated with bottom sediments which usually act as a sink for persistent chemicals (Forstner, 1987). Disturbance to sediments through natural occurrences such as burrowing or storms, or anthropogenic factors such as dredging increases the potential bioavailability of previously sediment bound contaminants. Bioconcentration of contaminants released from dredged sediments is known to occur (Seelye *et al.*, 1982) and presents a justifiable concern for management of contaminated sediments in the Tamar River. Any contaminants taken up by organisms directly may exert sublethal or lethal effects, therefore chemical analyses screening for presence of trace metals and organic compounds is necessary.

Significance of levels of trace metal contamination hinges on the uptake and sequestration of contaminants by organisms in their ambient environment. Trace metal uptake in most aquatic organisms involves a passive process occurring down a concentration gradient into the organisms tissues (Simkiss & Taylor, 1989) or in the case of particular metals, such as Cd, uptake may be linked to active ion pumps which function to transport major ions into and out of the organism. Bio-concentration of trace metals within the organism presents the potential for bioaccumulation within the ecosystem. Despite the role of several trace metals (Cr, Mn, Fe, Ni, Cu, Zn, Al) performing essential physiological functions (Simkiss & Taylor, 1989), any metals taken up by organisms may be toxic (Phillips, 1993), however their precise toxicity varies with species.

Various types of organic compounds are also considered to be of ecological importance with regard to toxicity. In contrast to trace metals, the bioaccumulation potential of trace

organic compounds is dependant on the hydrophobicity of the contaminant. Hydrophilic compounds present a higher toxic potential for aquatic organisms than hydrophobic ones. Hydrophobic compounds adsorbed to sediment also present a toxic hazard (Stegeman & Lech, 1991; Woodin *et al.*, 1997).

Methods

Trace metals analysis was conducted at the Central Science Laboratories; University of Tasmania. Heavy metals in pore water and sediments were analysed by ICP-MS (Inductively Coupled Mass Spectrometry (Finnigan - MAT Element)). Organic contaminants were analysed by Analabs (Hawthorn, Victoria) using GCMS (gas chromatography - mass spectrum).

Organics

Sediment was collected, as described in Section 2, and placed in acid washed glass containers. As samples were collected over a period of several weeks before analysis, they were preserved frozen. Analysis of pore water was not conducted due to the feasibility of collecting 2 L per sample as requested by Analabs. Four litres of fine sediment generally yields between 500 and 1,500 mL of pore water (Winger & Lasier, 1991), necessitating gross disturbance to sampling sites in achieving the volumes required for analysis.

Sediment for organics analysis was collected as described above, placed in acid washed glass storage containers and preserved frozen prior to transportation and analysis. Polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH) and benzene, toluene, ethyl benzene and xylene (BTEX) were analysed by Analabs (Hawthorn, Victoria) using gas chromatography - mass spectrum (GC-MS). Sediment samples were mixed with sodium sulphate, and dichloromethane/acetone was then added prior to each sample being sonicated and shaken, and the extract analysed.

*Trace Metals*Analysis of pore water involved collection of pore water by sipper technique (described in Section 2), using Teflon® tubing and acid-washed polymeric syringes. Samples stored in polymeric containers were frozen for storage and defrosted at +4°C prior to analysis. Samples were acidified with HNO_3, filtered and diluted by a factor of 25 before analysis. Values generated were the average of 10 scans over each element (dwell time for each element was 1 second). Blank values were subtracted from sample values.

Sediment core samples were collected using the procedure described in Section 2 and oven dried at 105°C overnight. A representative 0.5 g sample was taken and 2 mls concentrated HNO_3 added. The samples were left to digest for a number of days after which time the soil / acid mixtures were microwaved for 1-2 hours at a low power setting (low heat) to further the digestion process. The resultant mixture was diluted with 50 g of distilled water and internal standard added. All samples were filtered prior to ICP-MS analysis.

Results

Organics

Evidence of organics contamination (summarised in Table 3.1) was limited to three sites at Deceitful Cove and one at Squeaking Point. The frequency of detecting the same organic compounds from samples collected during the first and second surveys occurred twice and on both occasions were from sites at Deceitful Cove.

It is possible that of the twenty-three organic compounds analysed, many more than the nine detected would have been present in the sediment but were below detection limits. It is also possible that oxidation of anoxic pore waters could affect concentrations of some trace organic contaminants (Luoma & Ho, 1993). The results suggest that organic pollutants do not constitute a significant toxic load to the subtidal sediments of Port Sorell.

Table 3.1. Detection limits for surveyed organic compounds and presence of selected organic compounds detected at specific sites.

Compound	Site					Detection limit
	DC 1	DC2	DC3	DC4	SP3	mg kg^{-1}
PAHs (mg kg^{-1})						
Naphthalene						0.5
Acenaphthylene						0 5
Fluorene						0.5
Phenanthrene						0.5
Anthracene			1.2			0.5
Fluoranthene	2.3	1.0	1.3	4.7		0.5
Pyrene	1.7	0.7	1.0	1.0	3.3	0.5
Chrysene			0.5			0.5
Benzo (*a*) pyrene			1.4			0.5
Benzo (*b*) fluoranthene	0.5					0.5
Benzo (*k*) fluoranthene						0.5
Indeno (*1,2,3-cd*) pyrene						0.5
Dibenzo (*ah*) anthracene						0.5
Benzo (*ghi*) perylene						0.5
TPH/BTEX (mg kg^{-1})						
Hydrocarbons C6-C9						20
Hydrocarbons C10-C14	31					20
Hydrocarbons C15-C28	420		230			50
Hydrocarbons C29-C36						50
Benzene						0.1
Ethyl benzene						0.1
Toluene					0.2	0.1
Xylene						0.3

*Trace Metals*The highest levels of trace metals in both sediments and pore water were detected at the Tamar River locations. Deceitful Cove and East Arm exhibited significantly higher levels of sediment bound trace heavy metals compared to North East Arm and Port Sorell. Deceitful Cove sediment was significantly higher in Mn, Ni, Zn, Ag, Cd and Pb. Both East Arm and Deceitful Cove exhibited higher Cu levels than Squeaking Point and North East Arm (Table 3.2). Figure 3.1 graphically illustrates the range of concentrations found at each location with Deceitful Cove displaying the highest sediment trace metal loading overall.

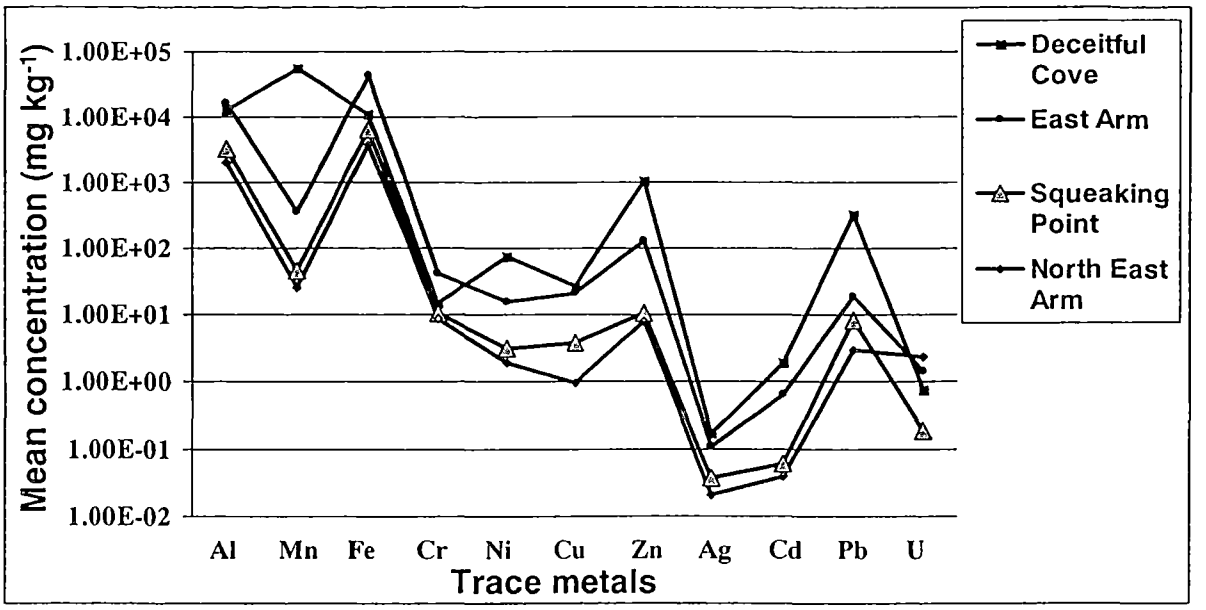


Figure 3.1. Trace metal concentration of sediment samples from survey locations in the Tamar river and Port Sorell estuaries

Table 3.2. Significant differences in sediment trace metal concentrations between locations. The degree to which the sediments found at each location are significantly different to other locations is represented by the number of asterix: *** p<0.0001, ** p<0.001, * p<0.05

Location	Al	Mn	Fe	Cr	Ni	Cu	Zn	Ag	Cd	Pb	Th	U
Deceitful Cove		*** EA SP NA			*** EA SP NA	*** SP NA	*** EA SP NA	*** EA SP NA	*** EA SP NA	*** EA SP NA		
East Arm	** DC SP NA		*** DC SP NA	*** DC SP NA		*** SP NA		* SP NA	* SP NA		*** DC	
Sqeaking Point												
North East Arm												

In comparison to the extreme range of concentrations and levels of significant differences detected in sediment analyses, trace metal concentrations detected in pore water exhibited a lower number of significantly different levels (Table 3.2). North East Arm exhibited the highest level of difference in pore water values, exceeding the levels of Fe, Cr, Cu and U found at other locations. Deceitful Cove displayed the highest levels of Mn and Ni. Figure 3.2 graphically illustrates the range of pore water trace metal concentrations detected at each location. Levels of Cd, Cr, Cu Pb, Ag, Th, Zn and Ni (excluding North East Arm sites) detected in pore water exceed the ANZEC Australian Water Quality Guidelines (1992) for marine waters.

Table 3.3. Significant differences in pore water trace metal concentrations between survey locations. The degree to which the pore waters found at each location are significantly different to other locations (location initials) is represented by the number of asterix: *** p<0.0001, ** p<0.001, * p<0.05

Location	Al	Mn	Fe	Cr	Ni	Cu	Zn	Ag	Cd	Pb	Th	U
Deceitful Cove		*** EA SP NA			* NA							
East Arm												
Sqeaking Point												
North East Arm			* DC EA SP	* DC EA		** DC EA						** DC EA SP

Nearly all pore water samples had precipitate present which is most likely to be Fe or Mn based species. Consequently even though the values of Fe and Mn are high (compared to the other elements) they may in fact be higher. Co-precipitation with other elements is also highly probable.

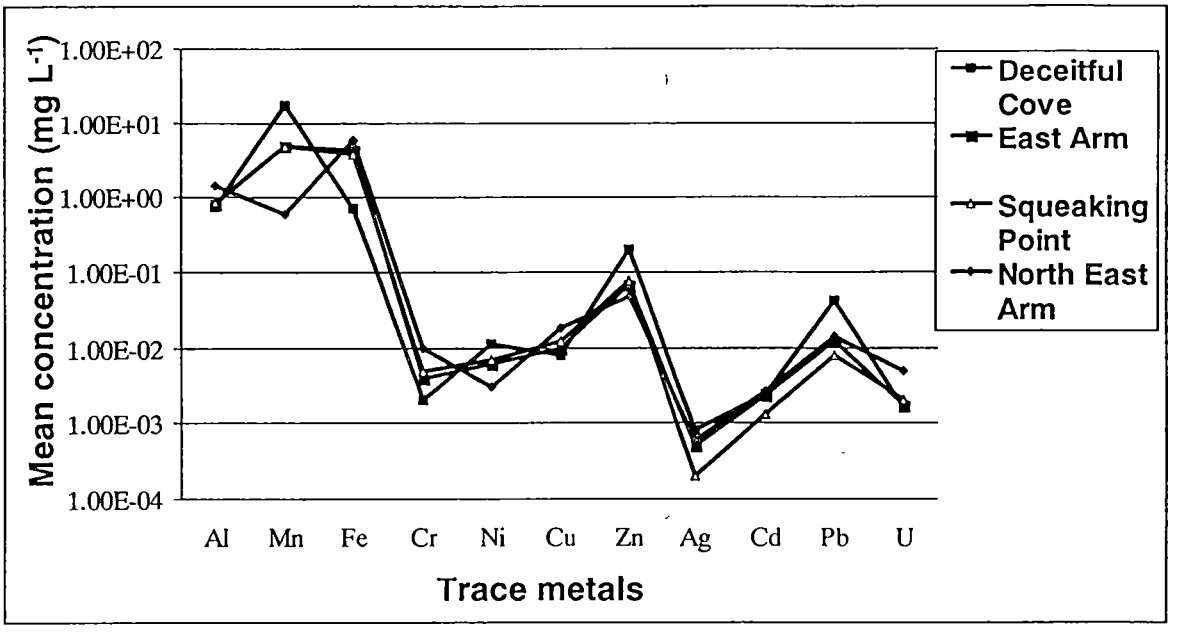


Figure 3.2. **Trace metal concentration of pore water samples from survey locations in the Tamar river and Port Sorell estuaries**

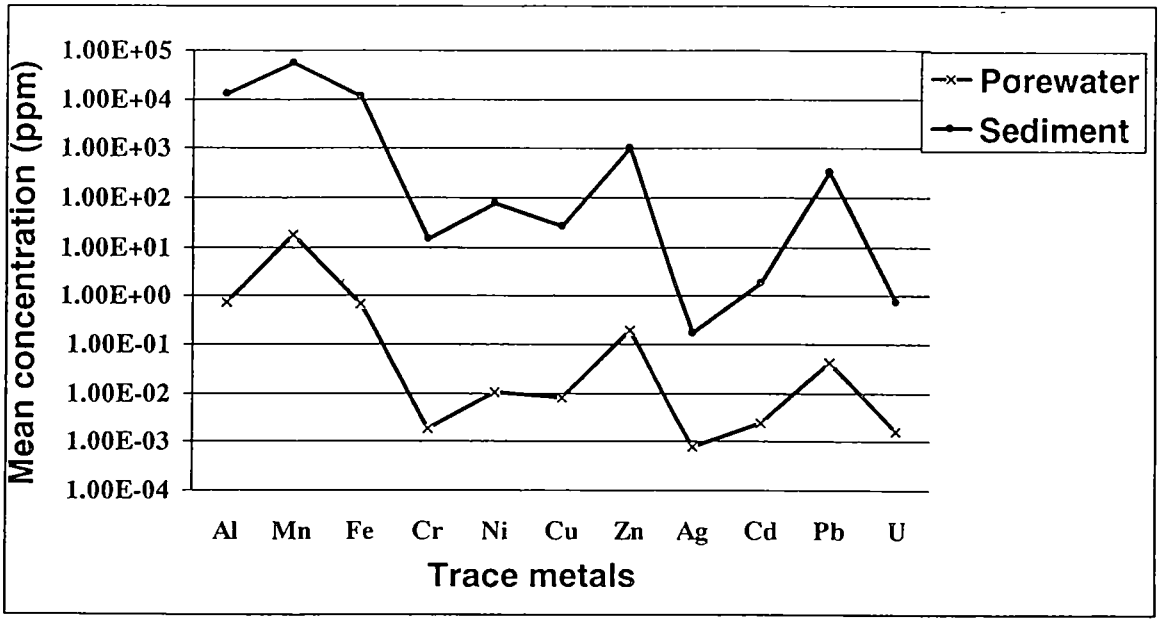


Figure 3.3. **Trace metal concentration of sediment and pore water samples from Deceitful Cove.**

Concentrations of trace metals detected in sediments far exceeded values detected in pore water samples. Figure 3.3 illustrates a dependency between sediment and pore water concentrations from Deceitful Cove sediments which is not unexpected. Many contaminants are known to become more strongly bound to sediments over time (SETAC-Europe, 1993) which would account for the appreciable difference between pore water and sediment values.

A similar pattern of chemical dependency was evident in pore water and sediment from the other locations, with some exceptions. Proportionally lower Mn levels were detected in pore water samples from East Arm, Squeaking Point and North East Arm. Proportionally lower Cd pore water levels were detected at Squeaking Point, and lower Cu and Cd levels from North East Arm pore water samples. The reason for this selective variation is unclear.

A one dimensional representation of the Euclidean distance of ranked metal contamination for each location, graphically illustrates the relative difference between chemical loading at Deceitful Cove relative to the less contaminated location at East Arm, and the two reference locations at Squeaking Point and North East Arm (Figure 3.4).

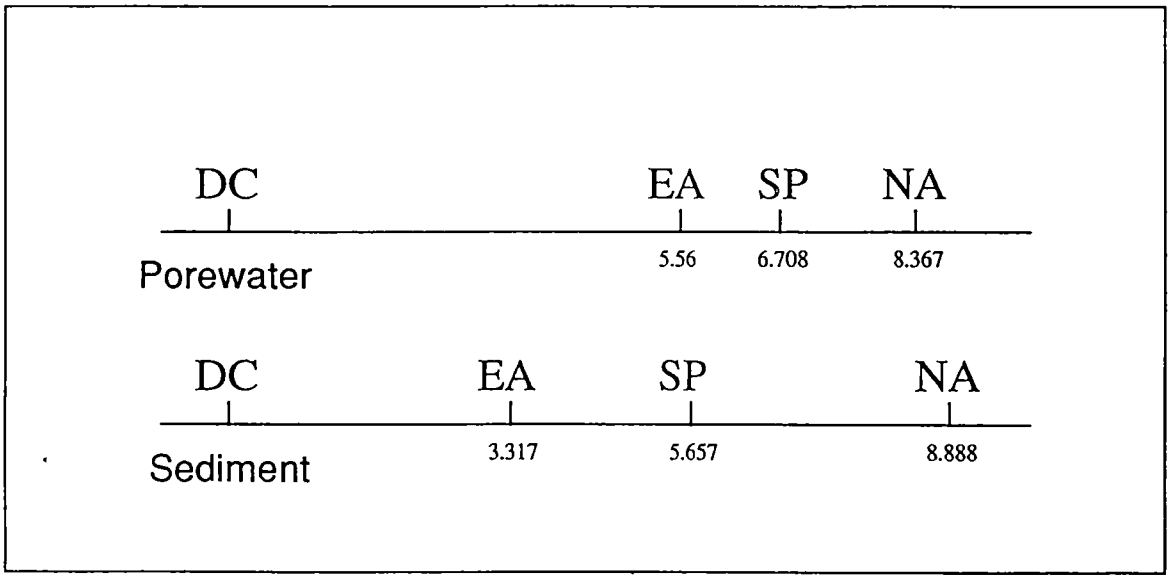


Figure 3.4. One dimensional representation of the Euclidean distance of ranked metal concentrations for each location: DC = Deceitful Cove, EA = East Arm, SP = Squeaking Point, NA = North East Arm.

SECTION FOUR

•

SUBTIDAL BIOTA

Introduction

Known contamination of sediments, together with poor benthic species diversity, can indicate a reduced sediment quality. Communities affected by storm activity or larval recruitment may display disturbed or reduced characteristics, however. In contrast to communities affected by pollution, the equilibrium condition of those communities is expected to return after a relatively short period of time (Gray, 1981). Assessment of sediment quality based on one-off short term benthic species surveys may be misleading. Furthermore establishing causality between affected ecosystems and pollutants is difficult without baseline data to work from.

A "level bottom" approach was used to investigate the assemblages of subtidal macro-invertebrates present at each site. This approach assumes the assemblages will be constant, and be related to the substratum on or in which they are found (Erwin, 1983). Selection of a 1 mm or 0.5 mm grid to delineate the macrofauna from meiofauna is arbitrary and subject to controversy. In general 1 mm screens are chosen to reduce the large sample variability generated by 0.5 mm screens picking up seasonal larval recruitment (Gray, 1981).
MethodSurface core samples were taken at each site (collection methodology described in Section 2). Each sediment core was carefully sorted using a 1 mm Endecott sieve with 0.2 µm filtered seawater. Specimens collected were preserved in 10% buffered formalin. All organisms were counted and identified to the lowest practical taxonomic level. Identification of species were confirmed by Dr Brian Smith, Queen Victoria Museum, Launceston, Tasmania, and Dr Graeme Edgar, School of Zoology, University of Tasmania.

Results and discussion

The number of species present at each location was not significantly different ($\chi^2 7=6.91$, $P>0.05$). However organism numbers differed between the first and second sampling period ($\chi^2 1=4.98$, $P<0.05$) displaying a trend towards an increase at East Arm, Squeaking Point and North East Arm (Figure 4.1).

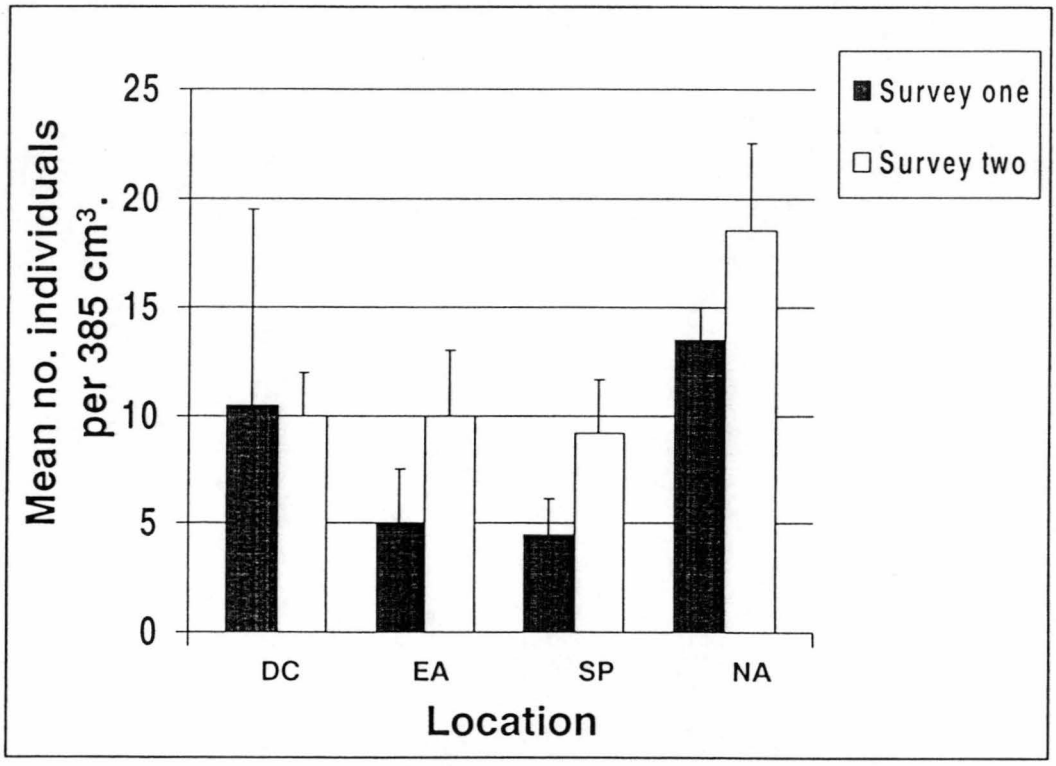


Figure 4.1. Variation in macroinvertebrate abundance over a two week period: DC=Deceitful Cove, EA=East Arm, SP=Squeaking Point, NA=North East Arm

Given the limitations of the narrow data set available, accounting for the apparent population difference of Deceitful Cove is not feasible at this stage, other than to tentatively suggest that either larval recruitment is inhibited at this site, or the populations present are exhibiting a perturbation response to an unknown geo-physicochemical event. The number of species present, relative to individual numbers overall (excluding the time factor), tentatively indicates that each location exhibits a uniform diversity in species composition with relatively few individuals representing each species (Figure 4.2). The

data are not unexpected in that low numbers of species are naturally found in estuaries (Wilson & Jeffrey, 1994). However, limited species numbers, in association with comparatively large populations, could be indicative of a response to polluted sediments. In undisturbed habitats larger organisms tend to dominate in terms of biomass (Warwick, 1986). Disturbance to that habitat generates a transfer of dominance to smaller opportunistic species which ultimately dominate in respect to numbers (Warwick, 1986).

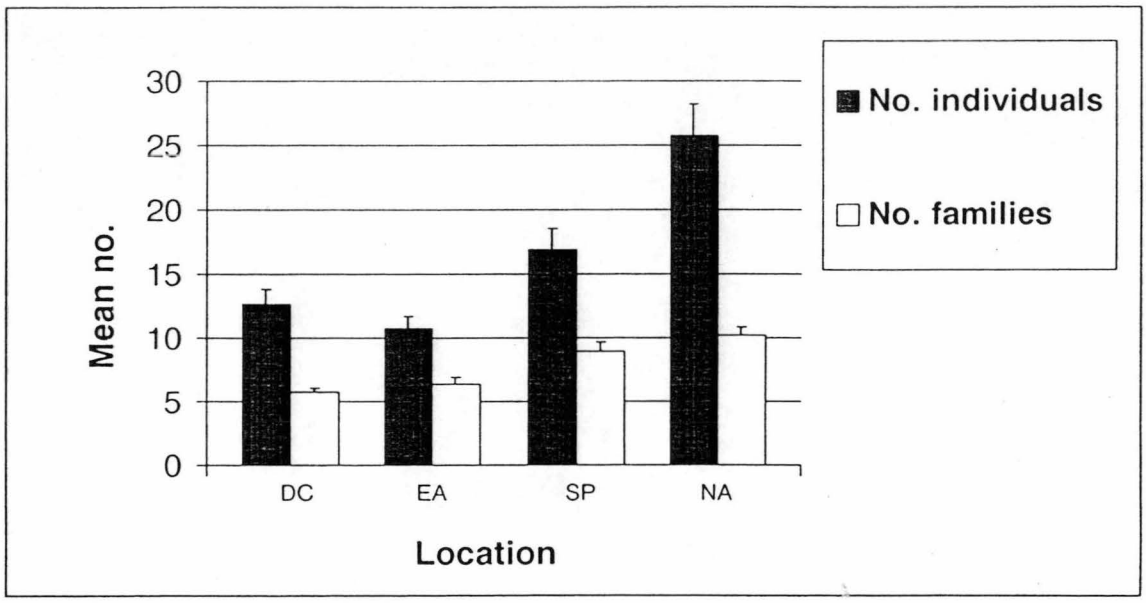


Figure 4.2. Variation in macroinvertebrate species richness and abundance over a two week period: DC=Deceitful Cove, EA=East Arm, SP=Squeaking Point, NA=North East Arm

A comparative study of species composition between locations would shed more light on the potential differences in subtidal biota. Until seasonal variation is accounted for, it is not valid to attempt a comparison based on the limited data set currently available. An increase in the number of replicate cores is advisable considering the apparent variability between sites, and overall low numbers of individuals and species recorded this season.

SECTION FIVE

•

GRANULOMETRY

Introduction

Particle size distribution is considered to be the most important physical variable when investigating community composition. The sediment grade influences a species success in colonising the substrate with respect to ease of burrowing and provision of suitable habitat for food organisms, for example. A high correlation between deposit grade and community composition and distribution patterns of particular species generally exists, assuming granulometry between locations is similar (Hartnoll, 1983). It is feasible (on the basis of physical factors) to assume the presence of a "characteristic" community at each site. Deviation from the norm could then indicate the presence of external factors over-riding the influence of the substratum on community structure.

A close correlation exists between interstitial oxygen content and deposit grade. As the particle size is reduced oxygen levels decrease. Coarse-grained sediments holding less pore water than fine-grained material, renew oxygen-depleted pore water at a greater rate through increased water movement within the sediment strata and exposure to surface waters. Subtidal sandy sediments are therefore able to support a relatively abundant and varied community of macro and meiofauna (Hartnoll, 1983). Aerobic macrofauna can exist below the redox-potential discontinuity in fine-grained sediments by employing siphons or digging permanent burrows to connect with the oxygenated layers at the surface. However, fine-grained anoxic sediments generally support reduced species diversity (Hartnoll, 1983).

Measurement of pore water content will also indicate the volume of water available to carry dissolved contaminates partitioned within whole sediment. Smaller grain size implies greater sediment surface area, thus increasing the potential for contaminant exchange between sediment particles and pore water.

Method

Pore water

Sediment cores stored in plastic containers were left to settle for several days, after which time any surface water present was carefully pipetted off. The samples were then removed from the containers, weighed, oven-dried at 110°C, and re-weighed.

Difference in weight after drying is indicated as evaporated pore water content. Hygroscopic water held to the surface of the sediment by capillary and adsorption forces is also evaporated during the drying process and is considered for the purposes of this study as a component of pore water (Mudroch & Bourbonniere, 1991). *Granulometry*Samples were air dried in a heated drying cabinet for 1-2 days. Oven drying was found to be unsuitable for grain size determination due to the formation of hard aggregates. Dried samples were placed in a graded Endecott sieve stack, and shaken. The volume of sediment left on each sieve was weighed and expressed as a percentage of the total sample weight.

Results and discussion

Granulometry

Particle size analysis identifies sediment from all locations as being predominantly fine sand. Deceitful Cove and East Arm exhibit a fine to slightly medium sand composition, whereas East Arm, and to a lesser extent Squeaking Point, show a fine to very fine sand composition (Figure 5.1). The sand to mud ratio identifies relatively uniform sand to mud ratios between all locations (Figure 5.2).

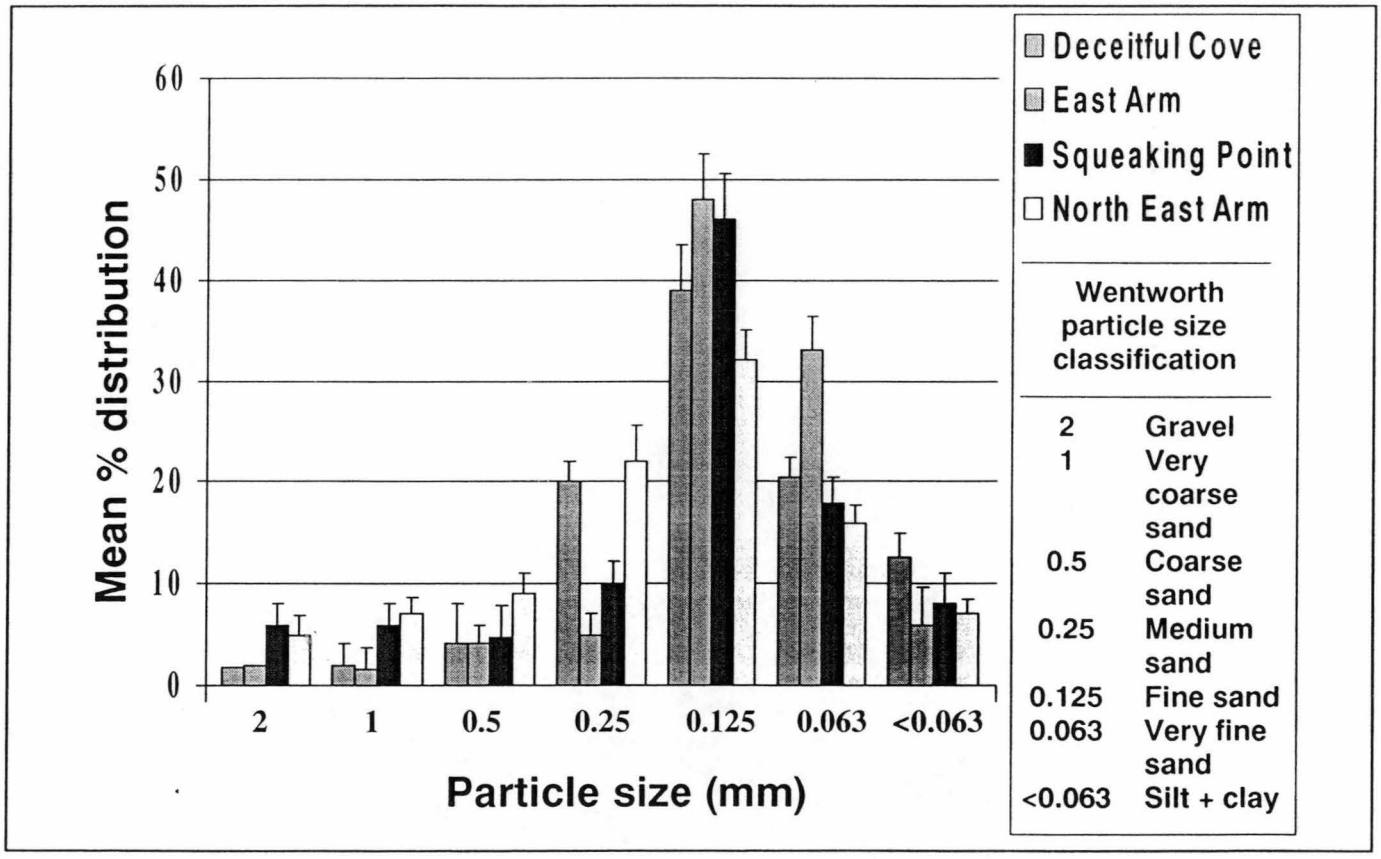


Figure 5.1. Particle size analysis of shallow subtidal sediment from survey locations in the Tamar River and Port Sorell estuaries.

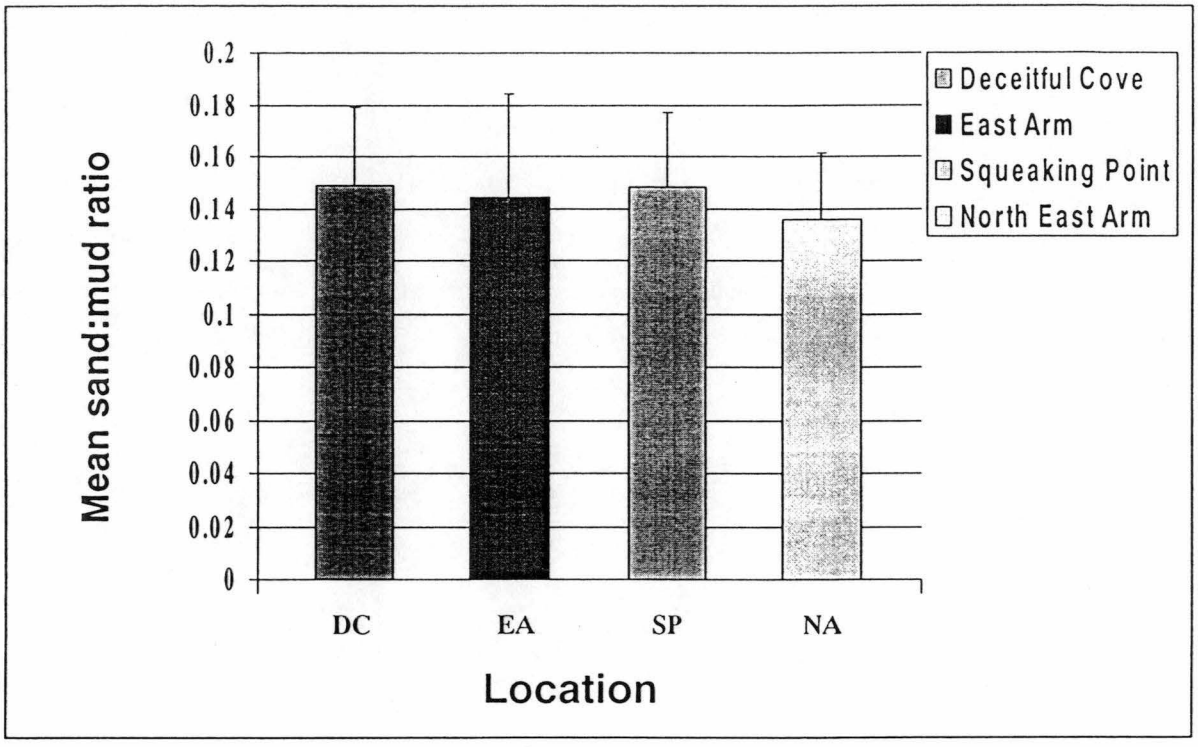


Figure 5.2. Sand:mud ratio of shallow subtidal sediment from survey locations in the Tamar River and Port Sorell estuaries.

Although the granulometry of each survey locations is similar, meiofauna and macrofauna larvae are particularly sensitive to particle size. Deposit feeders disturbing the sediment by movement and burrowing can cause surface instability producing a fine scale heterogeneity which may be significant to the local distribution of organisms (Hartnoll, 1983).

Pore water

Pore water content of sediment varied between locations (Figure 5.3). North East Arm sediment exhibited a significantly lower pore water content than Deceitful Cove and East Arm ($\chi^2 4=15.68$, $P<0.01$). Pore water content of surficial sediment layers at each of these locations may increase or decrease depending on disturbances such as bioturbation of the surface layers or changes in sedimentation processes.

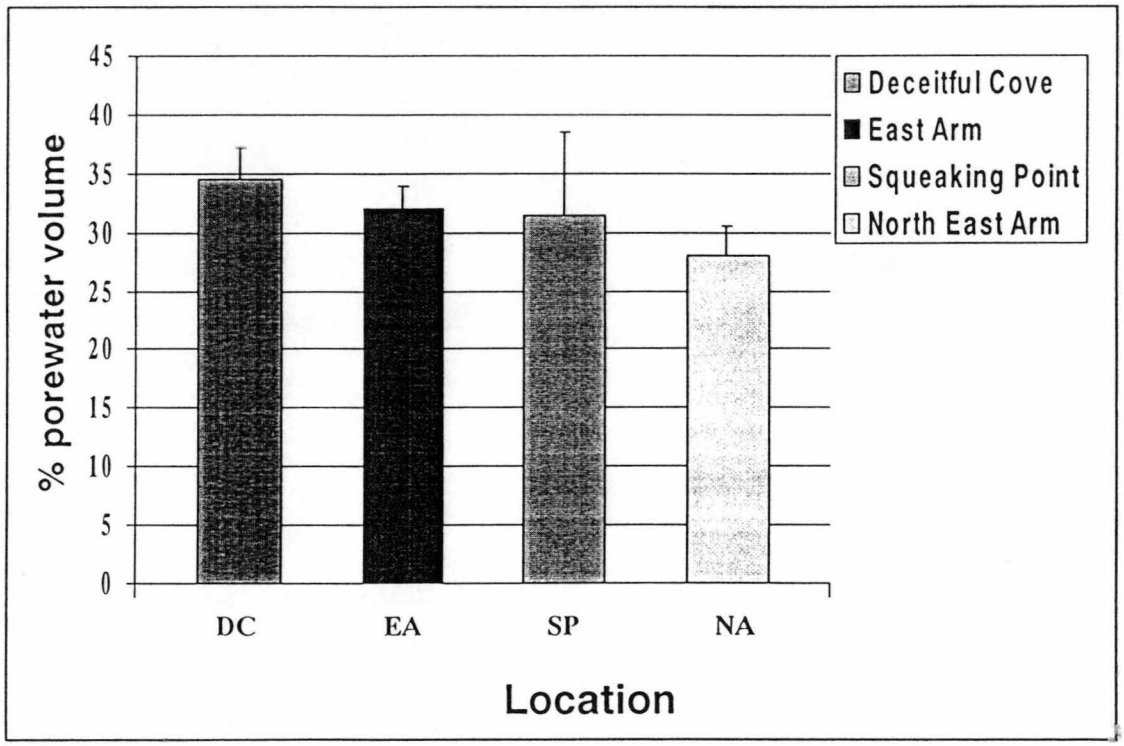


Figure 5.3. Pore water volume of shallow subtidal sediment from survey locations in the Tamar River and Port Sorell estuaries.

SECTION SIX

•

GENERAL DISCUSSION

The results from the preliminary survey have highlighted potential toxicity of whole sediments at Deceitful Cove and to a lesser extent East Arm. Indications are that toxic chemicals, principally trace metals, are bioavailable in the laboratory, but possibly not *in situ*. Both pore waters and sediment, aerated during toxicity tests, estimated the potential toxicity of uncovered or re-suspended sediments. rather than simulating *in-situ* conditions. Without evidence of alteration to the subtidal biota it is not possible at this stage to determine whether there is strong evidence for pollution-induced degradation at the surveyed locations in the Tamar River estuary.

Taking into account that sensitivity to contaminants may also be species specific, the bioassays chosen were valid in that the employment of a marine bacterium and phytoplankton test uptake of contaminants via common pathways in aquatic ecosystems. Sediment bioassays may be preferable to pore water for temporal analyses due to the lower variability from integration of contaminate levels over time. However, the difficulties associated with interpreting the sediment toxicity data, limits the usefulness of the solid phase assay in this situation.

Bioassays employed to screen for potential toxicity of sediment and pore water contaminants, in general supported the chemical analyses screening for occurrence of contamination. However, prediction of ecosystem change from bioassays alone presents a degree uncertainty in that it is difficult to extrapolate some of the effects of lifetime contaminant exposure from short-term bioassay exposures. Positive correlations are known to exist between toxicity levels recorded in benthic invertebrate bioassays and toxicity levels inhibiting benthic invertebrate colonisation of contaminated sediments (Giesy,

1990), for example. Field testing of bioassay results would strengthen the predictive power of the field survey component in this study.

Tentative agreement between the contaminant concentrations, toxicity tests and to a much lesser extent, community composition, points to the need for inclusion of macrofauna bioassays and longer-term exposure tests. Predictions of environmental impact would be enhanced by more representative exposure times to contaminants and pathways of uptake prevailing in the marine ecosystem.

Contaminants may remain most concentrated near the surface sediment or alternatively they may be leached down through the substrata resulting in increased contaminant concentration at depth. Bioturbation by infauna species can facilitate in the transportation of such contaminated sediments to the surface as well as providing a mechanism for vertical oxidation of sediments (Morrisey, 1995). The depth of the physical and biological turbation zone for each site is not known. Evidence of burrowing activity by crabs (*Heloecius cordiformis* and *Paragrapsus gaimardii*) and ghost *shrimps (Callianassa ceramica*) at Deceitful Cove in particular, points to potential re-suspension of contaminants *in situ*. Application of solvent-filled dialysis membranes (peepers) and gel probes could be used to investigate the historical depth profile of the sediments at this location, as it is likely that contaminants are present below the depth of 10 cm (Sodergren, 1990; Davidson & Zhang, 1994).

CONCLUSIONS

Subtidal sediment differed primarily in the concentrations of contaminants present and in bioassay response to exposure to sediments and pore water. Organic and trace metal contamination of Tamar River sediments exceeded levels detected at Port Sorell. Whereas pore water bioassays reflected chemical contamination at far lower levels than than for sediments, the difficulties associated with interpreting Microtox® sediment bioassay data limit the precision of estimates of toxicity to one which is very tentative. Relatively low species abundance and diversity were detected at all locations, with species abundance at Deceitful Cove reflecting a possible response to environmental conditions not present at other locations. The inherent limitations associated with short-term survey data, necessitates longer term field and experimental research to effectively determine the quality and ecological impact of Deceitful Cove subtidal sediments.

REFERENCES

Anderson, J., Birge, W., Gentile, J., Lake, J. & Rogers, J. J. (1987). Biological effects. Bioaccumulation and ecotoxicology of sediment associated chemicals. In K. L. Dickson, A. W. Maki, & W. A. Brung (Eds.), *Fate and effects of sediment-bound chemicals in aquatic systems* . New York: Pergamon Press.

Ankley, G. T., Hoke, R. A., Giesy, J. P.& Winger, P. V. (1989). Evaluation of the toxicity of marine sediments and dredge spoils with the Microtox bioassaay. *Chemosphere, 18*, 2069-2075.

Carr, R. S. & Chapman, D. C. (1995). Comparison of methods for conducting marine and estuarine sediment pore water toxicity tests - extraction, storage and handling techniques. *Archives of Environmental Contamination and Toxicology, 28*, 69-77.

Carr, R. S., Williams, J. W. & Fragata, C. T. B. (1989). Development and evaluation of a novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus*. *Environmental Toxicology & Chemistry, 8*, 533 - 543.

Chapman, P. M. (1989). Review: current approaches to developing sediment quality criteria. *Environmental Toxicology & Chemistry, 8*(589-599).

Chapman, P. M. (1990). The Sediment Quality Triad approach to determining pollution-induced degradation. *Science of the Total Environment, 97/98*, 815-825.

Chapman, P. M., Wang, F., Janssen, C., G., P. & Allen, H. E. (1998). Ecotoxicology of metals in aquatic sediments: binding and release, bioavailability , risk assessment, and remediation. *Canadian Journal of Fisheries & Aquatic Science, 55*, 2221-2243.

Davidson, W. & Zhang, H. (1994). In situ speciation measurements of trace compounds in natural waters using thin-film gels. *Nature, 367*,546-548.

Erwin, D. G. (1983). The community concept, *Sublittoral ecology. The ecology of the shallow sublittoral benthos* (pp. 144-164). Oxford: Clarendon Press.

Forstner, O. (1987). Sediment associated contamination - an overview of scientific basis for developing remedial options. *Hydrobiologia, 149*, 221-246.

Gawne, K. M. & Richardson, B. J. (1992). *An investigation into the distribution and accumulation of selected chemical species in the waters, sediments and biota of the lower Tamar estuary, Tasmania* : Deakin University Centre for Biological and Chemical Research.

Giesy, J. P. (1990). Benthic invertebrate bioassays with toxic sediment and pore water. *Environmental Toxicology & Chemistry, 9*, 233-248.

Gray, J. S. (1981). *The ecology of marine sediments*. Oxford: Cambridge University Press.

Hartnoll, R. G. (1983). Substratum. In R. Earll & D. G. Edwin (Eds.), *Sublittoral ecology. The ecology of the shallow sublittoral benthos* (pp. 97-124). Oxford: Clarendon Press.

Luoma, S. N. (1990). Processes affecting metal concentrations in estuarine and coastal marine sediments. In R. W. Furness & P. S. Rainbow (Eds.), *Heavy metals in the marine environment* (pp. 51-56). Boca Raton: CRC Press.

Luoma, S. N.& Ho, K. T. (1993). Appropriate uses of marine and estuarine sediment bioassays. In P. Calow (Ed.), *Handbook of ecotoxicology* (Vol. 2, pp. 193-226). London: Blackwell Scientific Publications.

Miedecke, J. (1992). *Deceitful Cove rehabilitation study. Final Report.* : Meidecke and Partners.

Morrisey, D. (1995). Estuaries. In A. J. Underwood & M. G. Chapman (Eds.), *Coastal marine ecology of temperate Australia* (pp. 152-171). Sydney: University of New South Wales press.

Mudroch, A. & Bourbonniere, R. A. (1991). Sediment sampling, handling and processing. In A. Mudroch & S. D. Macknight (Eds.), *CRC Handbood of techniques for aquatic sediment sampling* (pp. 131-170). Boston: CRC Press.

Phillips, D. J. H. (1993). Bioaccumulation. In P. Calow (Ed.), *Handbook of Ecotoxicology* (Vol. 2, pp. 378-396). London: Blackwell Scientific Press.

Ringwood, A. H., DeLorenzo, M. E., Ross, E. R. & Holland, F. (1997). Interpretation of Microtox Solid Phase toxicity tests: The effects of sediment composition. *Environmental Toxicology and Chemistry, 16*, 1135-1140.

Seelye, J. G., Hesselberg, R. J. & Mac, M. J. (1982). Accumulation by fish of contamination released from dredged sediments. *Environmental Science & Technology, 16*, 459-463.

SETAC-Europe. (1993, 8-10 November 1993). *SETAC Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments.* Paper presented at the Society of Environmental Toxicology and Chemistry - Europe, Slot Moermond Congrecentrum Renesse, The Netherlands.

Simkiss, K. & Taylor, M. G. (1989). Metal fluxes across the membranes of aquatic organisms. *Review of Aquatic Science, 1*, 173-188.

Skacel, A., Riha, V., Radclavsky, K., & Hlavata, M. (1993). The use of the Microtox system for evaluation of toxicity of wastes in Vitkovice Steelworks, Ostrave (Czechoslovakia)., *The Science of the Total Environment.* (Vol. Supplement 1993, pp. 195-205). Amsterdam.: Elsevier Science Publishers B.V.

Sodergren, A. (1990). Monitoring of persistant lipophilic pollutants in water and sediment by solvent-filled dialysis membranes. *Ecotoxicology & Environmental Safety, 19*, 143-149.

Stauber, J. & Florence, T. M. (1985). Interactions of copper and manganese: a mechanism by which manganese alleviates copper toxicity to the marine diatom *Nitzchia closterium*. *Aquatic Toxicology, 7*, 241-254.

Stauber, J. L. (1995). Toxicity testing using marine and freshwater unicellular algae. *Australian Journal of Ecotoxicology, 1*, 15-24.

Stegeman, J. J. & Lech, J. J. (1991). Cytochrome p-450 monooxygenase systems in aquatic species: carcinogen metabolism and biomarkers for carcinogen and pollutant exposure. *Environmental Health Perspectives, 90*, 101-109.

Swartz, R. C. (1987). Toxicological methods for determining the effects of contaminated sediment on marine organisms. In K. L. Dickson, A. W. Maki, & W. A. Brung (Eds.), *Fate and effects of sediment-bound chemicals in aquatic systems* . New York: Pergamon Press.

Troup, B. N., Bricker, O. P. & Bray, J. T. (1974). Oxidation effect on the analysis of Fe in the interstitial water of recent anoxic sediments. *Nature, 249*, 237-239.

Warwick, R. M. (1986). A new method for detecting pollution effects on marine macrobenthic communities. *Marine Biology, 92*, 193-200.

Wilson, J. G. & Jeffrey, D. W. (1994). Benthic biological pollution indices in estuaries. In J. M. Kramer (Ed.), *Biomonitoring of Coastal Waters and Estuaries.* (pp. 311-327). Boca Raton: CRC Press.

Winger, P. V. & Lasier, P. J. (1991). A vacuum-operated pore water extractor for estuarine and freshwater sediments. *Archives of Environmental Contamination and Toxicology, 21*, 321-324.

Woodin, B. R., Smolowitz, R. M. & Stegman, J. J. (1997). Indiction of cytochrome P4501A in the intertidal fish *Anoplarchus purpurescens* by Prudhoe Bay crude oil and environmental induction in fish from Prince William Sound. *Environmental Science and Technology, 31*(4), 1198-1205

EFFECTS OF STORAGE TEMPERATURE AND TIME ON SEDIMENT AND PORE WATER TOXICITY

Introduction

Due to logistical constraints it is not always possible to conduct field sampling and laboratory based bioassays on samples within the same day. If storage of sediments and pore waters are expected to exceed 24 hours, the effect of storage on toxicity of the contaminated and reference media should be determined. Toxicity of pore water extracted from sediment stored at +4°C is known to exhibit significant short-term changes over several days to weeks (Carr & Chapman, 1995). Other studies have documented either little change in toxicity of sediments stored at +4°C over several weeks (Redmond *et al.*, 1996), decreased toxicity evident only after several months (Ciarelli *et al.*, 1998), or a marked increase in toxicity over several weeks to months at storage temperatures of +4°C and -22°C (Dillon *et al*, 1994). Assessment of the direction and degree of change in toxicity is further complicated by differences in sensitivity of test species to contaminated media (Becker & Ginn, 1995). These studies highlight the unpredictable nature of changes in toxicity with storage over time and temperature, and the need to assess the effect of storage on the sediment of interest.

The aim of this experiment was two-fold. Firstly, to determine if toxicity of sediment and pore water samples extracted from local contaminated sediment changes over time. Secondly, to determine the optimum storage conditions of sediment and pore waters with respect to temperature. The direction and degree of change in toxicity was assessed using two bioassays, the *Nitzschia closterium* algal growth inhibition test (Stauber *et al.*, 1994) and the Microtox® Solid Phase test (Microbics Corporation).

Materials and methods

Whole sediment samples were collected from Deceitful Cove on the Tamar River estuary, reference sediment samples were obtained from North East Arm, Port Sorell estuary (Deceitful Cove Preliminary Subtidal Survey, Figure 1 , Appendix 1).

Sediment collection, extraction and storage

Sediments were collected from Deceitful Cove (contaminated) in the Tamar River estuary, and North East Arm (reference) in the Port Sorell estuary. Whole sediment samples were collected at the lowest tide using 70 mm diameter benthic core tubes inserted 10 cm into the soft substrate (collection methodology decribed in detail *in* Deceitful Cove Preliminary Subtidal Survey, Appendix 1). Sample cores were pooled and stored in cooled, sealed, light-shielded containers for transportation back to the laboratory. The time taken to collect sediment and transport it to the laboratory was less than 2 hours. Ten litres of sediment was collected from each location. Whole sediment was stored at +4°C and - 20°C for up to 6 weeks. For logistical reasons reference sediment from North East Arm was tested over a three week time frame, as opposed to six weeks for contaminated sediment.

Vacuum-operated pore water extraction (sipper technique) was used to collect pore water for algal bioassays (Winger & Lasier, 1991). Pore water was extracted immediately on arrival at the laboratory (+4°C), centrifuged at 2500 rpm (664 G) (Carr & Chapman, 1995), then stored in acid-washed Nalgene containers at +4°C and -20°C. Deceitful Cove pore water was stored up to 6 weeks, North East Arm water was stored up to 3 weeks.

Bioassays

The *Nitzschia closterium* algal growth inhibition test and Microtox® Solid Phase test were used to detect changes in pore water and sediment toxicity respectively. Both techniques are described in full *in* Deceitful Cove Preliminary Subtidal Survey (Appendix 1).

Physicochemical Parameters

Ammonia, pH, redox potential and salinity of pore water were measured immediately prior to testing: pH and redox potential of pore water samples were measured by an Activon 209 pH/mV meter; salinity was determined using a Shibuya Optical S-10 salinity refractometer; and un-ionised ammonia (NH_3-N) was determined by the salicylate method using a Hach - DR/2000 spectrophotometer.

Chemical analysis

Trace metal analysis was conducted at the Central Science Laboratories, University of Tasmania. Heavy metals in sediments were analysed by ICP-MS (Inductively Coupled Mass Spectrometry (Finnigan- MAT Element)). Organic contaminants were analysed by Analabs (Hawthorn, Victoria) using GC-MS (Gas chromatography- mass spectrum).

Statistical analyses

Algal growth inhibition EC_{50} values were calculated using the Dynamic Energy Budget model (DEB) computer package DEBtox (Kooijman & Bedaux, 1996). Microtox® Solid Phase EC_{50} values were calculated by the trimmed Spearman-Karber method, using the ToxCalc computer package (ΣTidepool Scientific Software). Statistical comparisons between toxicity data were determined by analysis of variance (ANOVA) using the computer package JMP (SAS Institute Inc.,1995). Assumptions of ANOVA were tested using Shapiro-Wilk K and Cochran's test for normality and homogeneity of variances respectively. If significant differences were detected, the Tukey-Kramer comparison of means test was used.

Results

Microtox

Week 0 indicates toxicity data measurements after an initial 24 hour storage. Microtox® Solid Phase analysis of Deceitful Cove sediments exhibited an increase in EC_{50} values over time, indicating a continuing decrease in sediment toxicity when stored at -20°C (Figure 1). Storage at +4°C resulted in little change in toxicity over the first three weeks, subsequent to a marked decrease after that time (Figure 1).

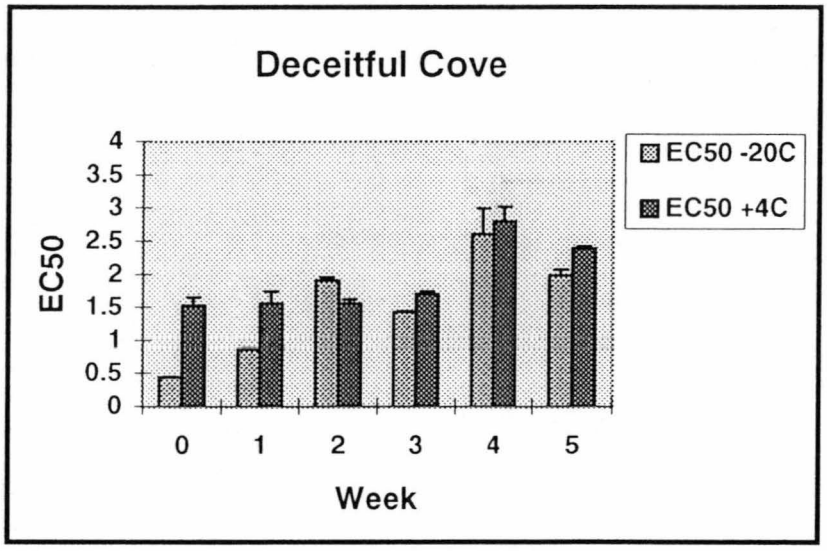


Figure 1. Temporal variation in Microtox EC_{50} [mean % wt. of sample per vol.diluent] values of Deceitful Cove sediment stored at +4°C and -20°C

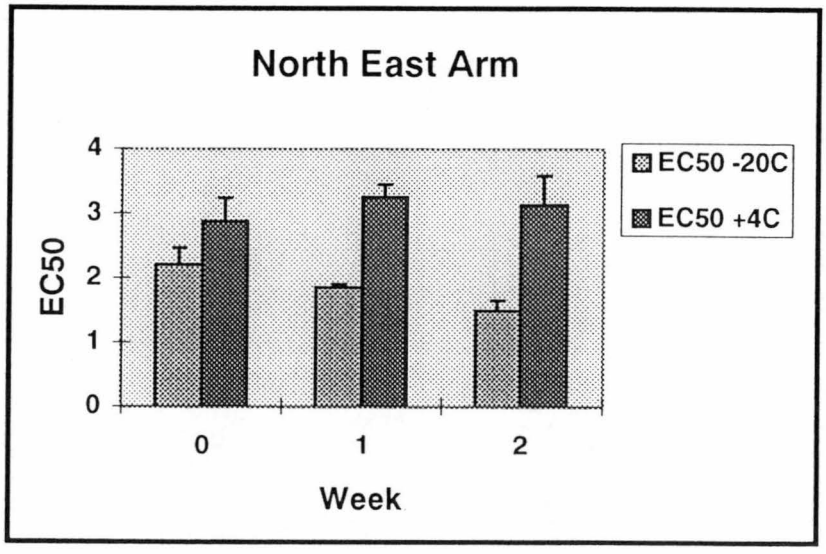


Figure 2. Temporal variation in Microtox EC_{50} [mean % wt. of sample per vol. diluent] values of North East Arm sediment stored at +4°C and -20°C.

North East Arm (reference sediment) indicated an increase in sediment toxicity over time when stored at -20°C (Figure 2). Toxicity of North East Arm sediment stored at +4°C remained stable over time (Figure 2).

Algal growth inhibition

EC_{50} values for Deceitful Cove pore waters remained relatively stable at -20°C, whereas storage at +4°C elicited a decrease in toxicity over 5 weeks (Figure 3).

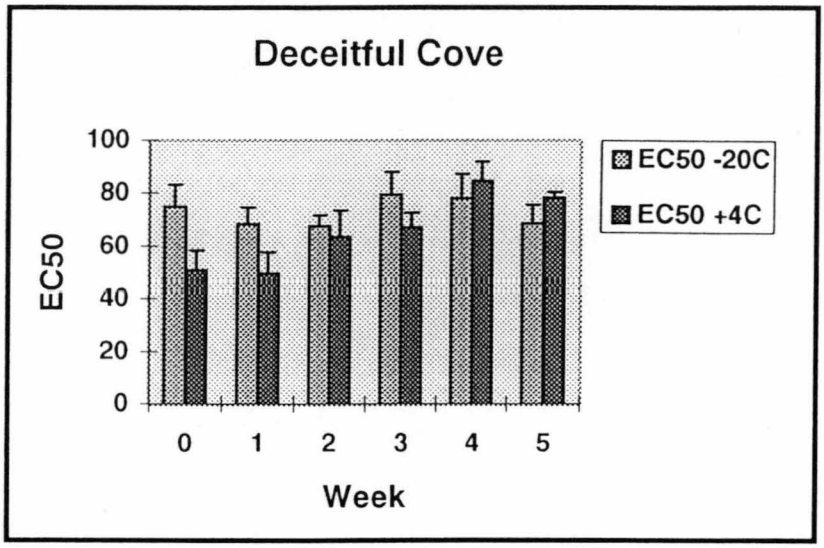


Figure 3. Temporal variation in Algal growth inhibition EC_{50} [% pore water] values for Deceitful Cove pore water stored at +4°C and -20°C.

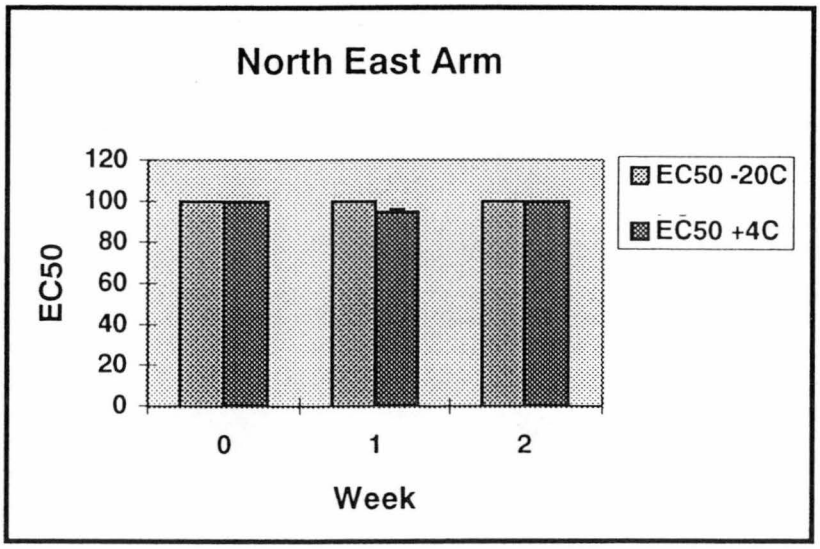


Figure 4. Temporal variation in Algal growth inhibition EC_{50} [% pore water] values for North East Arm pore water stored at +4°C and -20°C.

North East Arm exhibited EC50 values exceeding 100 for both storage temperatures, indicating comparatively non-toxic pore water relative to Deceitful Cove (Figure 4).

pH

A trend towards an increase in alkalinity of Deceitful Cove and North East Arm pore water was present over the first two weeks of storage (Figures 5 & 6). Pore water stored at -20°C exhibited a greater increase in alkalinity compared to +4°C, for both Deceitful Cove and North East Arm pore waters (Figures 5 & 6).

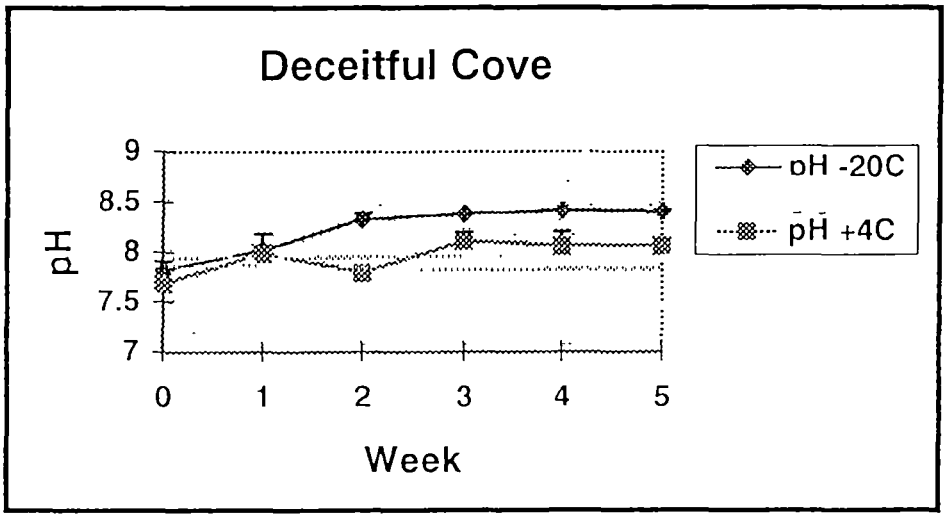


Figure 5. Temporal variation in pH of Deceitful Cove pore water stored at +4°C and -20°C.

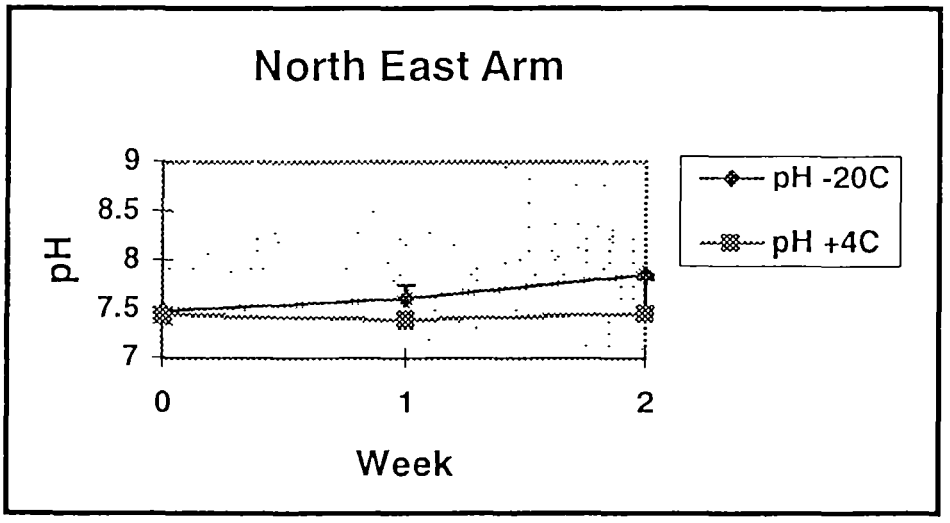


Figure 6. Temporal variation in pH of North East Arm pore water stored at +4°C and -20°C.

Ammonia

Pore water ammonia levels were consistently higher in samples stored at -20°C compared to those stored at +4°C, with the exception of week 5, when a marked increase in ammonia was recorded. Both Deceitful Cove and North East Arm exhibited a peak in pore water ammonia concentrations at week 1 (Figure 7 & 8).

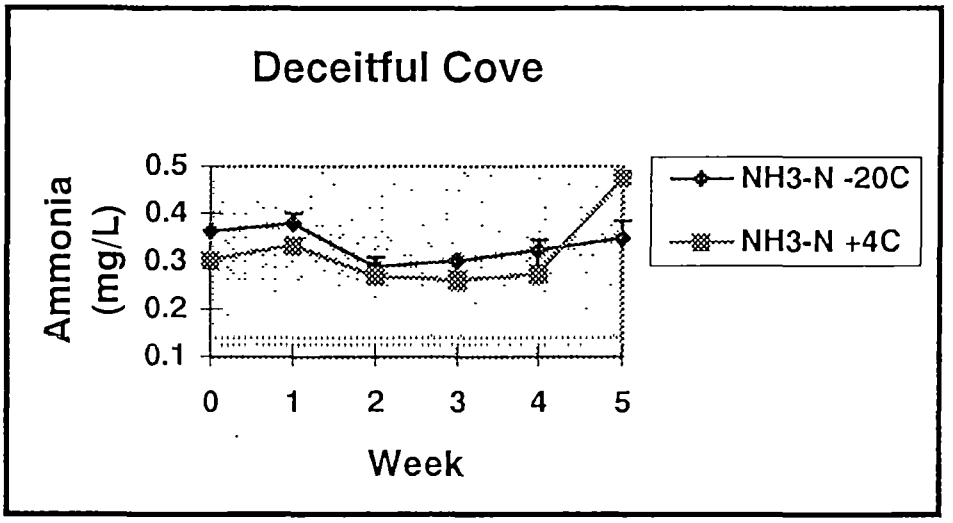


Figure 7. Temporal variation in ammonia (NH_3-N L^{-1}) concentration of Deceitful Cove pore water stored at +4°C and -20°C.

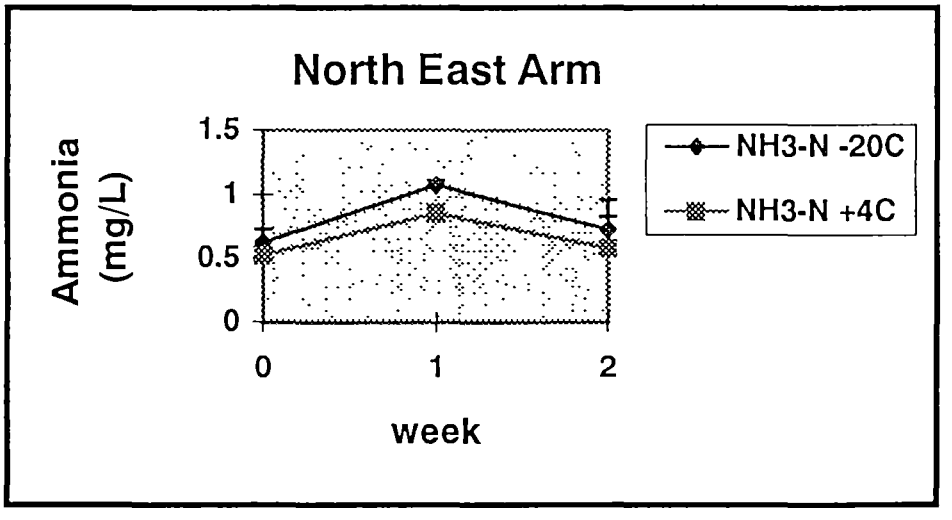


Figure 8. Temporal variation in ammonia (NH_3-N L^{-1}) concentration of North East Arm pore water stored at +4°C and -20°C.

Redox potential

Changes in redox potential of pore waters were generally lower at -20°C than +4°C (Figures 9 & 10). Deceitful Cove exhibited a marked peak in redox potential at week 1, followed by a general decline. After five weeks storage, the redox potential of Deceitful Cove pore water had increased 6.4% when stored at +4°C, and decreased 7.1% at -20°C (Figure 9).

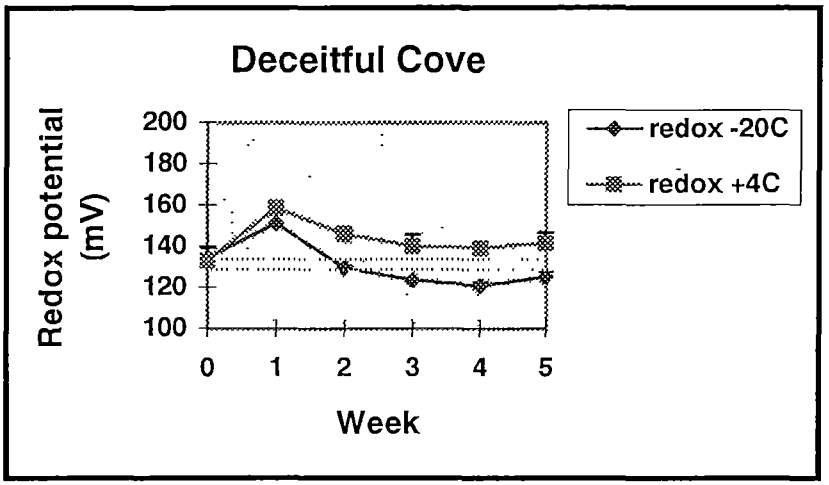


Figure 9. Temporal variation in redox potential [mV] of Deceitful Cove pore water stored at +4°C and -20°C.

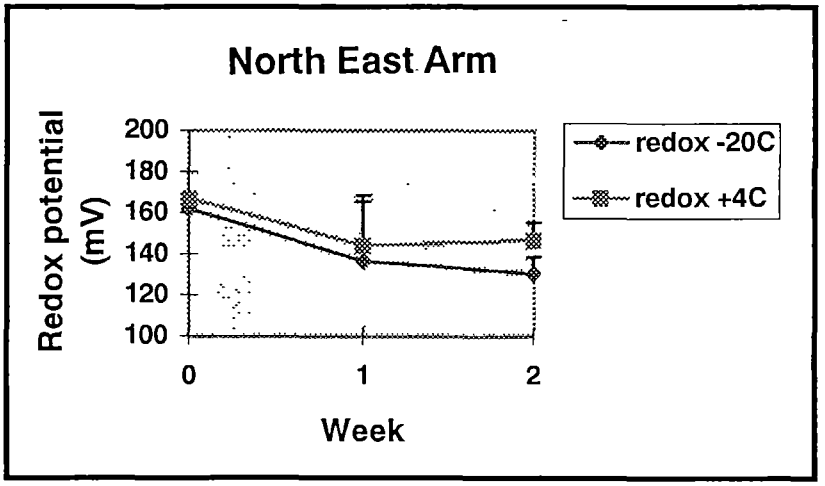


Figure 10. Temporal variation in redox potential [mV] of North East Arm pore water stored at +4°C and -20°C.

North East Arm exhibited an overall decline in redox potential in pore water over 2 weeks, resulting in a decrease of 19.4% at storage of -20°C and 12% at storage of +4°C (Figure 10).

Salinity

Storage temperature did not affect pore water salinity. Salinity remained stable throughout the course of the experiment indicating little, if any, evaporation had occured. Deceitful Cove exhibited lower salinity levels than North East Arm (Table 1).

Table 1. Mean salinity of pore water (±SE) from Deceitful Cove and North East Arm stored at -20°C and +4°C.

Storage temperature	Deceitful Cove	North East Arm
-20°C	30.7 ‰ ± 1.46	32.3 ‰ ± 0
+4°C	30.3 ‰ ± 0.55	32.3 ‰ ± 0

Chemical Analyses

Trace metal and organic contaminant concentrations were higher in Deceitful Cove sediments, relative to North East Arm sediments. Deceitful Cove sediments contained 9 polycyclic aromatic hydrocarbon (PAH) species above the detection limit of 0.5 mg kg^{-1} (dry weight) (Table 2).

Table 2. PAH concentrations in subtidal marine sediment collected from Deceitful Cove and North East Arm.

Polycyclic aromatic hydrocarbons (PAHs)	Deceitful Cove (mg $kg^{-1)}$	North East Arm (mg $kg^{-1)}$
Fluoranthene	1.88	<0.5
Pyrene	2.1	<0.5
Benzo[*a*]anthracene	1.00	<0.5
Chrysene	1.18	<0.5
Benzo[*b*]fluoranthene	1.4	<0.5
Benzo[*k*]fluoranthene	0.53	<0.5
Benzo[*a*]pyrene	0.82	<0.5
Indeno[*123-cd*]pyrene	0.77	<0.5
Benzo[*ghi*]perylene	0.63	<0.5

No detectable levels of PAHs were found in North East Arm pore water. With the exception of Fe, trace metal concentrations were higher in Deceitful Cove sediments (Table 3).

Table 3. Trace metal concentrations in subtidal marine sediment collected from Deceitful Cove and North East Arm.

Trace metal	Deceitful Cove ($mg\ kg^{-1}$)	North East Arm ($mg\ kg^{-1}$)
Ag	0.194	0.007
Al	11812.8	2123.6
Cd	2.712	0.117
Cr	15.79	7.6
Cu	34.11	0.89
Fe	11555.2	13224.9
Mn	59938.5	28.4
Ni	141.69	3.09
Pb	397.7	3.07
Sn	0.69	0.61
Zn	1167.96	16.22

Particle size distribution

Deceitful Cove exhibits higher silt, and lower clay levels than North East Arm (Table 4). Additionally, Deceitful Cove exhibits a total organic carbon content which is approximately two thirds higher than the level detected in North East Arm sediment.

Table 4. Size distribution of sediment particles expressed as percentages (dry weight).

Sediment characteristic	Deceitful Cove	North East Arm
Sand	74.20 %	86.02 %
Silt	6.98 %	4.52 %
Clay	3.14 %	10.56 %
Total organic carbon	15.68 %	5.53 %

Discussion

Toxicity of contaminated (Deceitful Cove) and non-contaminated (North East Arm) sediments and pore waters exhibited alteration in levels of toxicity as a function of storage time and temperature. Pore water toxicity appears comparatively stable at -20°C for both contaminated and non-contaminated media, whereas storage at +4°C results in decreased toxicity of contaminated pore water over time. Sediments exhibit little change in toxicity when stored at +4°C. At -20°C the toxicity of contaminated sediment decreases, however, whereas the toxicity of non-contaminated sediment increases.

The results from this study concur with those of (Naudin *et al.*, 1994) who found that during refrigeration (+4°C) of contaminated pore waters, toxicity decreases over time, whereas sample toxicity remains relatively stable when samples are frozen. Elevated ammonia levels in pore water samples after week 1, indicates possible microbial denitrification occuring within pore water samples, and may be due to inadequate filtering of particulate matter by the glass air stone. However, the elevation in ammonia concentration and redox potential, resulting in a potential increase in bioavailability of metals from fine sediment particles (Zhuang & Allen, 1994) during the first week of storage, was not sufficient to elicit a marked increase in algal growth inhibition.

The decrease in toxicity of contaminated pore water stored at +4°C signifies the need to either process samples immediately or freeze them. The recorded shift towards a pH of 8.5, is representative of pH conditions in seawater where precipitation of metals as hydroxides is likely to occur, thereby reducing the exposure of metals in solution to the test algae. Trace metal composition of pore water is an important factor in the toxicity of *N.closterium*, due to the interaction between metals. Stauber and Florence (1985) found that copper toxicity in *N. closterium* is reduced in the presence of Fe(III) coatings on the surface of the cell wall. Presence of other trivalent metal ions such as Al, Fe and Cr, and divalent metals (Co and Mn) that can be oxidised to trivalent species, also provide protection against copper toxicity by forming a layer of hydrated metal(III)oxide around the cell, absorbing copper before it can penetrate into the cell (Stauber & Florence, 1987).

Additionally, organic chelators present in the pore waters, such as humic substances, can ameliorate the toxicity of a wide range of toxic metal complexes provided that the ligand itself is not toxic (Stauber & Florence, 1985; Florence *et al.*, 1992).

The direction and magnitude of change in toxicity of frozen sediments, relative to the stablility of refrigerated samples, indicates long term storage of frozen samples is not recommended. Theoretically, an increase in sediment toxicity could be due to an increase in ammonia, and or hydrogen sulphide, or a decrease in oxygen concentration. High levels of ammonia in sediment can potentially confound interpretation of toxicity test results by masking the toxicity of other contaminants present (Ankley *et al.*, 1989; Moore, *et al.*, 1997). However, *Vibrio fischeri* (Microtox®), is not sensitive to NH_3-N (Ankley *et al.*, 1990; Naudin *et al.*, 1994), indicating other factors are likely to be responsible for the increased toxicity in reference sediment stored at -20°C. Although pore water is removed by centrifugation prior to *Vibrio fischeri* exposure to sediments, the reduction in redox potential recorded in pore water samples indicates the potential for anoxic conditions, and hydrogen sulpfide, to develop within sediment samples. It is possible the microbial populations of the contaminated sediment are less robust to freezing than populations in reference sediment, possibly resulting in elevated activity in reference sediment when thawed. It is unlikely the clay and silt content of North East Arm sediment would be altered during freezing, thereby reducing the likelihood of a decreased light production output resulting from bacterial adhesion to fine sediment particles (Ringwood *et al.*, 1997). The increase in toxicity of reference sediment presents an interesting problem that warrants further investigation.

Conclusions

Effects of storage time and temperature on sediment and pore water vary between contaminated and non-contaminated media. To reduce the potential for spurious toxicity results to occur, it is advisable the following storage procedures are recommended for Deceitful Cove (contaminated) and North East Arm (reference) sediments and pore waters. Pore water should be frozen immediately after extraction as toxicity remains relatively stable when stored at -20°C. Sediments should be refrigerated and processed as soon as possible as sediment toxicity remains relatively stable when stored at +4°C up to 3 weeks.

References

Ankley, G.T., Hoke, R.A., Giesy, J.P. & Winger, P.V.(1989). Evaluation of the toxicity of marine sediments and dredge spoils with the Microtox bioassay. *Chemosphere, 18,* 2069-2075.

Ankley, G. T., Katko, A. & Arthur, J. W. (1990). Identification of ammonia as an important sediment-associated toxicant in the Lower Fox River and Green Bay, Wisconsin. *Environmental Toxicology and Chemistry, 9*(313-322).

Becker, D. S. & Ginn, T. C. (1995). Effects of storage time an toxicity of sediments from Puget Sound, Washington. *Environmental Toxicology and Chemistry, 14*(5), 829-835.

Carr, R. S. & Chapman, D. C. (1995). Comparison of methods for conducting marine and estuarine sediment pore water toxicity tests - extraction, storage and handling techniques. *Archives of Environmental Contamination and Toxicology, 28*, 69-77.

Ciarelli, S., Vonck, W. A. P. M. A., Vanstraalen, N. M. & Stronkhorst, J. (1998). Ecotoxicology assessment of contaminated dredged material with the marine amphipod *Corophium volutator. Archives of Environmental Contamination and Toxicology, 34*(4), 350-356.

Dillon, T. M., Moore, D. W. & Jarvis, A. S. (1994). The effects of storage and temperature and time on sediment toxicity. *Archives of Environmental Contamination and Toxicology, 27*(1), 51-53.

Florence, T. M., Powell, H. K. J., Stauber, J. L. & Town, R. M. (1992). Toxicity of lipid-soluable copper(11) complexes to the marine diatom *Nitzchia closterium* amelioration by humic substances. *Water Research, 26*, 1187-1193.

Kooijman, S. A. L. M. & Bedaux, J. J. M. (1996). *The analysis of aquatic toxicity data.* Amsterdam: VU University Press.

Moore, D. W., Bridges, T. S., Gray, B. R. & Duke, B. M. (1997). Risk of ammonia toxicity during sediment bioassays with the estuarine amphipod *Leptocheirus plumulosus*. *Environmental Toxicology and Chemistry, 16*, 1020-1027.

Naudin, S., Garric, J., Vindimian, E., Bray, M., Migeon, B., Vollat, B. & Lenon, G. (1994). Influence of the sample preservation mode to assess the chronic toxicity of an industrial effluent. *Ecotoxicology and Environmental Safety, 30*, 54-62.

Redmond, M. S., Crocker, P. A., McKenna, K. M., Petrocelli, E. A., Scott, K., J. & Demas, C. R. (1996). Sediment toxicity testing with the amphipod *Ampelisca abdita* in Calcasieu estuary, Louisiana. *Archives of Environmental Contamination & Toxicology,30(1)*, 53-61.

Ringwood, A. H., DeLorenzo, M. E., Ross, E. R. & Holland, F. (1997). Interpretation of Microtox Solid Phase toxicity tests: The effects of sediment composition. *Environmental Toxicology and Chemistry, 16*, 1135-1140.

Stauber, J. L. & Florence, T. M. (1985). The influence of iron on copper toxicity to the marine diatom, *Nitzchia closterium* (Ehrenberg) W. Smith. *Aquatic Toxicology, 6*, 297-305.

Stauber, J. L. & Florence, T. M. (1987). mechanisms of toxicity of ionic copper and copper complexes to algae. *Marine Biology, 94*, 511-519.

Stauber, J. L., Tsai, J., Vaughan, G. T., Peterson, S. M. & Brockbank, C. I. (1994). *Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills* (National Pulp Mills Research Program Technical Report Series No.3): CSIRO Division of Coal and Energy Technology Centre for Advanced Analytical Chemistry.

Winger, P. V. & Lasier, P. J. (1991). A vacuum-operated pore water extractor for estuarine and freshwater sediments. *Archives of Environmental Contamination and Toxicology, 21*, 321-324.

Zhuang, Y., Allen, H.E. & Fu, G. (1994). Effect of aeration of sediment on cadmium binding. *Environmental Toxicology & Chemistry, 13,* 717-724.

TAXONOMIC RESOLUTION OF BENTHIC MACROINVERTEBRATE FAUNA

Introduction

The current debate over the level of taxonomic resolution required to detect changes or losses of marine or estuarine biodiversity is unresolved. Identification to species level is considered to be a major time and cost constraint which may be alleviated by lower taxonomic resolution of collected samples (Warwick, 1993). Several studies (Gray *et al* 1990; Aagard, 1993; Warwick 1994) have used identification to taxonomic levels higher than species to detect environmental disturbances in soft bottom macrobenthos. These studies provide evidence that using higher levels may remove variablility at the lower taxonomic levels which might obscure community patterns. If disturbance effects are picked up at the highest taxonomic levels, the detection of impact at the class or phylum level implies a functional coherence of species within each higher taxon (Warwick, 1993). It is conceivable that physiological traits might be synapomorphies for any clade (phylum to genus), and so the whole clade would exhibit a broadly similar response to a physiological challenge.

A fundamental criticism levelled at the use of higher taxonomic resolution is that much of the knowledge of how organisms function is at the species level and may not be relevant to all other members of that genus or family (Hutchings, 1999). The potential exists for significant differences even between populations of the same species. Hutchings (1999) also asserts that higher taxonomic groups have no biological meaning, whereas the species level provides a suite of information regarding the ecology, physiology and biology of those organisms. Additionally, Hutchins (1999) states that without species data it is inappropriate to compare the fauna of different areas or same area over time. However, the RIVPACS (River Invertebrate Prediction and Classification System) (Wright, 1995) approach, which is used in Australia, the United Kingdom and South Africa, and the AusRivAS (Australian River Assessment Scheme) (Smith *et al.*, 1999) system, use family level classification to generate site-specific predictions of macroinvertebrate fauna expected in the absence of environmental stress. The predicted faunal assemblage is

compared with the observed assemblage to evaluate the ecological conditions of rivers. Both systems are currently used in Australia to generate national and regional classifications of rivers, and are able to discriminate between sites based on the macroinvertebrate fauna classified to family level.

While acknowledging the potential for loss of information associated with aggregation of species to higher taxonomic levels, Warwick (1986) suggests that taxonomic resolution should be matched to the objectives of the study. Without *a priori* knowledge, a pilot study is necessary to determine the taxonomic resolution required. If the question is whether a difference exists between polluted and reference sites, then the difference will most likely be picked up at various levels of taxonomic resolution (Warwick, 1986). Environmental variation or disturbance is likely to affect assemblages at the level of species whereas anthropogenic disturbance effects are clearly evident at higher levels because anthropogenic disturbances are assumed to be stronger (Warwick, 1988a; Warwick, 1988b; Warwick, 1988c). However, this generalisation may not hold true for all benthic subtidal communities (James *et al.*, 1995).

The objective of this study is to determine at what level of taxonomic resolution differences between sites can be detected, in order to facilitate future biomonitoring of northern Tasmanian estuaries.

Method

Shallow subtidal benthic macroinvertebrate data from the Sediment Quality Triad (Chapter 2), were used to determine the taxonomic resolution required to detect spatial differences between benthic assemblages in northern Tasmanian estuaries. Estimates of diversity (Shannon-Wiener *H'*) at species, family, class and phylum levels were determined using the software package PRIMER v4 (Plymouth Routines in Multivariate Ecological Research) developed at the Plymouth Marine Laboratory (Clarke & Warwick, 1994). A two-dimensional non-metric multidimensional scaling (MDS) ordination based on Bray-Curtis similarity matrices (Bray & Curtis, 1957) of fauna in all samples was also performed

to display relationships among samples of organisms in multi-dimensional space. Rare species, whose occurrence at a particular site may largely be due to chance, were excluded by removal of species accounting for <3% of the total abundance in any one sample (Clarke & Warwick, 1994). Statistical comparisons between diversity measures were determined by analysis of variance (ANOVA) using the computer package JMP (SAS Institute Inc.,1995). If significant overall differences were detected, the Tukey-Kramer comparison of means test was used.

Results and discussion

Diversity measures indicate a marked difference between estuaries at both the species and family levels, but differentiation between the estuaries is lost at the phylum level (Figure 1).

Aggregation of samples from species to higher taxonomic levels indicates that MDS ordinations of assemblages at the family level are comparable to a full species analysis (Figure 2). Ordination of the class level data indicates a clear separation between estuaries, and between Squeaking Point and North East Arm, but no distinct partition between Deceitful Cove and East Arm is apparent. Differentiation between, but not within estuaries, is retained at the phylum level. Differentiation between Deceitful Cove and East Arm at the phylum level is markedly reduced.

The results indicate that aggregation of species to the family level is comparable to a full species analysis when using Shannon Weiner diversity measures and MDS ordinations to differentiate between contaminated and reference sites. If the fauna is classified only to phylum level, the analysis runs the risk of failing to detect real spatial differences between assemblages.

Defining diversity indices at hierarchical taxonomic levels for internal comparative purposes is valid, though not commonly practised (Clarke & Warwick, 1994). The similarity between species and family diversity in this study is not surprising considering

the high proportion of families containing only one species (Appendix 4a). This means that the present data set may not provide a good indicator of the effect of aggregating from species to family.

Ordinations of marine macrobenthic assemblages from field studies elsewhere have demonstrated similar results to this study. Studies of oil contaminated sediments in the North Sea, and the Bay of Morlaix, France (Gray *et al.*, 1990; Clarke, 1993), and trace metal contaminated sediments at Port Pirie in South Australia (Clarke, 1993), showed that ordination analysis at different taxonomic resolutions generated distribution patterns associated with pollutant gradients as clearly as analysis at species level. Unlike this study, the assemblage pattern in these studies was distinguishable up to the phylum level. The absence of a clear community response at the highest taxonomic level (Warwick, 1988b) may be a function of the degree of contamination to which the organisms are exposed, or possibly the absence of a clear gradient between the survey sites, as samples were not, as in the case studies referred to above, collected along transects at increasing distances from the point source.

It is also possible that the degree of data transformation used may influence the ordination pattern at the highest taxonomic level. The species data from the North Sea, Bay of Morlaix and Port Pirie studies were either moderately ($\sqrt{}$) or severely transformed ($\sqrt{\sqrt{}}$), whereas the data from this study were not. MDS ordinations using Bray-Curtis similarities of species abundance data collected from contaminated sediments in Loch Linnhe, Scotland (Pearson, 1975), displayed little difference between 4^{th} root transformed and non-transformed data aggregated to the family level (Clarke & Warwick, 1994). However, the pattern differed between the two sets of data at the phylum level. Transformed data maintained the pattern shown at the family level, whereas the non-transformed data did not: differentiation between groups of sites became less clear. The alteration in assemblage pattern above family level in non-transformed Loch Linnhe data, is very similar to the findings from this study.

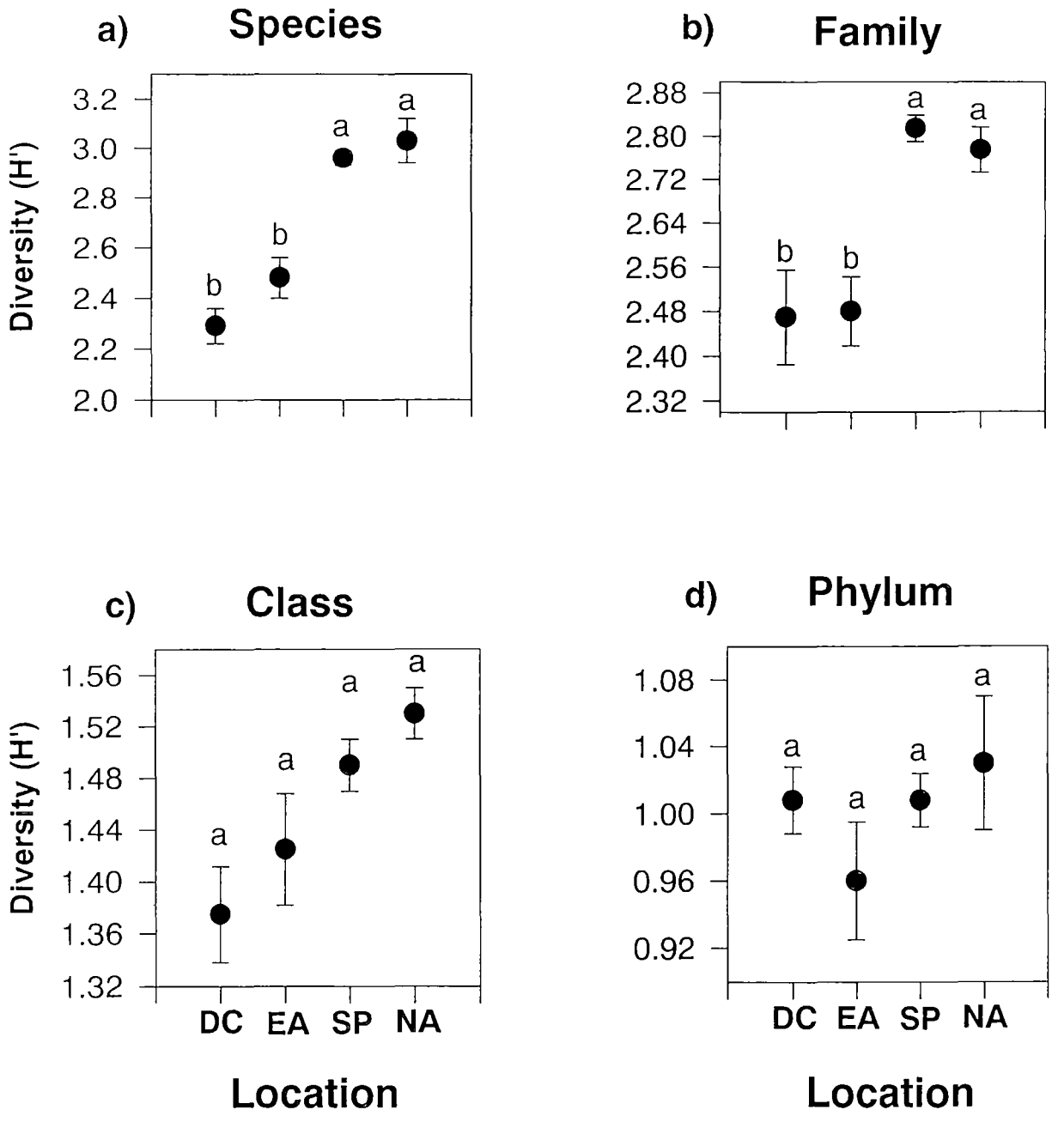


Figure 1. Shannon Weiner measures of diversity at the species, family, class and phylum level.

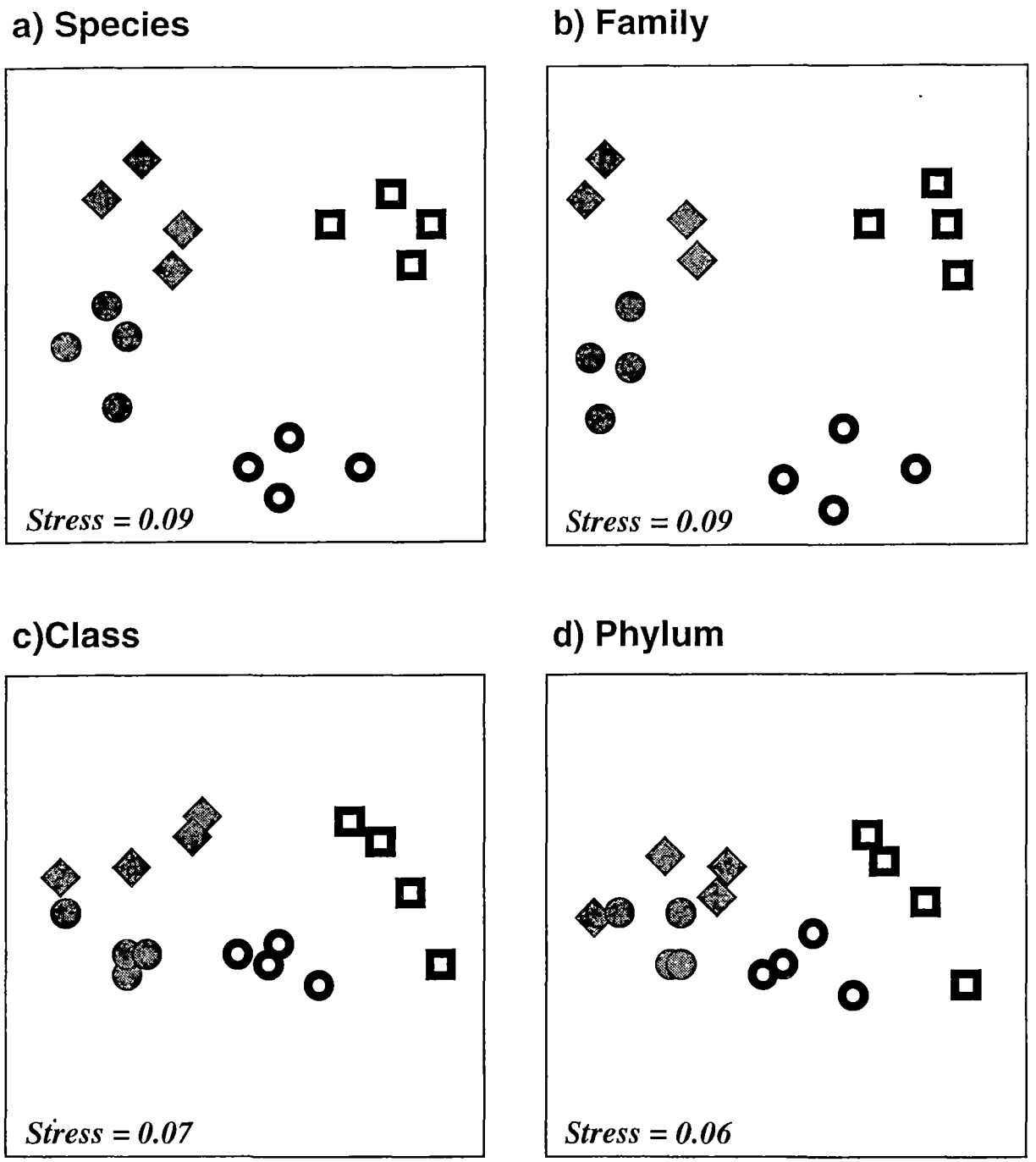


Figure 2. **MDS ordinations of the 4 survey locations (16 sites) based on species, family, class and phylum abundance's, and Bray-Curtis similarities: ◆ Deceitful Cove, ● East Arm, ○ Squeaking Point, □ North East Arm.**

Identifying organisms to family level is an attractive option for extended monitoring programs. It offers a considerable a saving in time, particularly if the taxonomic expertise required to identify organisms to species level is not readily available. Additionally, access to accurate, detailed taxonomic keys is a another limiting factor. For some organisms, identification is very difficult below family level, particularly where the species may not have been described, or identification is not based on morphological structure readily observable with the aid of a standard optical microscope.

Strictly speaking, the data from the SQT is not a full species analysis, as several species were not identified to species level, but only to family (Appendix 4a). However, a full species analysis is desirable as the current survey of fauna allows a database for the two estuaries in question to be added to in the future, and this may not be possible if the fauna are only sorted to higher taxonomic levels (Hutchings, 1999). This leaves the option open for aggregation of the species data to be performed at a later stage if required, for example, for a comparison with another, less well-resolved, study. The communities of the Tamar and Port Sorell estuaries are relatively simple, so the time and cost constraints normally associated with species level identification are not excessive. Further, identification to species level allows detailed analysis of benthic assemblages in order to identify species indicative of certain environmental conditions, or which discriminate between environmental conditions. In addition to higher taxonomic level identification, the use of indicator species offers a practical option for future monitoring by non-specialists.

References

Argard, J. B. R., Gobin, J. & Warwick, R. M. (1993). Analysis of marine macrobenthic community structure in relation to pollution, natural oil seepage and seasonal disturbance in a tropical environment. *Marine Ecology Progress Series, 92*, 233-243.

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs, 27*, 325-349.

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology, 18*, 117-143.

Clarke, K. R. & Warwick, R. M. (1994). *Change in marine communities: an approach to statistical analysis and interpretation.* Plymouth: Plymouth Marine Laboratory.

Gray, J. S., Clarke, K. R., Warwick, R. M. & Hobbs, G. (1990). Detection of initial effects of pollution on marine benthos: an example from the Ekofisk and Eldfisk oilfields, North Sea. *Marine Ecology Progress Series, 66*, 285-299.

Hutchings, P. (1999). Taxonomy of estuarine invertebrates in Australia. *Australian Journal of Ecology, 24*, 381-394.

James, R. J., Lincoln Smith, M. P. & Fairweather, P. G. (1995). Sieve mesh-size and taxonomic resolution needed to describe natural spatial variation of marine macrofauna. *Marine Ecology Progress Series, 118*, 187-196.

Pearson, T. H. (1975). The benthic ecology of Loch Linnhe and Loch Eil, a sea-loch system on the west coast of Scotland. IV. Changes in the benthic fauna attributable to organic enrichment. *Journal of Experimental Marine Biology and Ecology, 20*, 1-41.

Smith, M.J., Kay, W.R., Edward, D.H.D., Papas, P.J., Richardson, K.S., Simpson, J.C., Pinder, A.M., Cale, D.J., Horwitz, P.H.J., Davis, J.A., Yung,F.H., Norris, R.H. & Halse, S.A. (1999). AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwater Biology.41(2),* 269-282.

Warwick, R. M. (1986). A new method for detecting pollution effects on marine macrobenthic communities. *Marine Biology, 92*, 193-200.

Warwick, R. M. (1988a). Analysis of community attributes if the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. *Marine Ecology Progress Series, 64*, 167-170.

Warwick, R. M. (1988b). The level of taxonomic discrimination required to detect pollution effects on marine benthic communities. *Marine Pollution Bulletin, 19*, 259-268.

Warwick, R. M. (1988c). Effects on community structure of a pollution gradient-summary. *Marine Ecology Progress Series, 46*, 207-211.

Warwick, R. M. (1993). Environmental impact studies on marine communities: Pragmatical considerations. *Australian Journal of Ecology, 18*, 63-80.

Wright, J.F. (1995). Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Australian Journal of Ecology, 20(1),*181-197.

APPENDIX 4A.

Species list of macroinvertebrates collected in cores from Deceitful Cove and East Arm in the Tamar River estuary, and Squeaking Point and North East Arm in the Port Sorell estuary.

Foraminifera
- Foraminiferan unidentified (1 species)

Porifera
- Poriferan unidentified (1 species)

Cnidaria
- Anthozoa
  - Actinaria
    - Anemone unidentified (1 species)

Nemertea
- Nemerteans unidentified (4 species)

Annelida
- Oligochaeta (1 species)
  - Oligochaete unidentified (1 species)
- Polychaeta
  - Amphionomidae *Eurythoe sp.*
  - Arenicolidae unidentified (1species)
  - Capitellidae *Heteromastus sp.*
  - Cirratulidae unidentified(1 species)
  - Dorvilleidae unidentified(1 species)
  - Eunicidiae unidentified (1 species)
  - Glyceridae unidentified(1 species)
  - Lumbrinereidae *Lumbrineris sp.*
  - Magelonidae *Magelona sp*
  - Maldanidae *Maldane (?) sp.*
  - Nephtyidae *Nephtys australiensis*
  - Nereidae *Neranthes sp*
    - unidentified (1 species)
  - Orbinidae *Scoloplos simplex*
    - unidentified (1species)
  - Orpheliidae *Armandia intermedia*
  - Phyllodocidae unidentified(1species)
  - Polynoidae unidentified(3 species)
  - Spionidae unidentified(1species)
  - Trichobranchidae *Terebellides sp.*
    - unidentified (1 species)

Mollusca
- Bivalvia
  - Assimineidae *Assiminea buccinoides*
  - Erycinidae *Lasaea australis*
  - Glycymeridae *Glycymeris flammeus*?
  - Laternulidae *Laternula tasmanica*
  - Lucinidae *Epicodakia tatei*
  - Mactraridae *Mactra rutescens*
  - Mesodesmatidae *Paphies ercinaea*

Montacutidae *Mysella donacformis*
Mytilidae *Xenostrobus inconstans*
Semelidae *Theora? lumbrica*
Tellinidae *Tellina deltoidalis*
Tellina marginata
Tellina botanica
Veneridae *Eumarcia fumigata*
Katelysia peronii
Katelysia rhytiphora
Katelysia scalarina

Gastropoda
Prosobranchia
Amphibolidae *Salinator fragilis*
Buccinidae *Nassarius (Niotha) pauperatus*
Nassarius (Zeuxis) pyrrhus
Cylinidae *Tornatina exserta*
Hydrobiidae *Tatea rufilabris*
Littorinidae *Bembicium melanostoma*
Littorina (Austrolittorina) unifasciata
Pyramidellidae *Turbonilla (Pyryiseus) fusca*?
Retusidae *Retusa pelyx*
Turbinidae *Pseudoliotia micans.*

Arthropoda
Crustacea
Ostracoda
Ostracod unidentified (1 species)
Malacostraca
Cumacea
Culinacea *Cycluspis caprella*
Tanaidacea
Leptochelia (?) dubia
Amphipoda
Gammaridae *Gammaropsis sp.*
Leptochelia dobia?
Birubus sp.
unidentified (2 species)
Decapoda
Callianassidae *Callianassa ceramica*
Callianassa sp.2
Grapsidae *Paragrapsus gaimardii*
Mysidacea unidentified (2 species)
Ocypodidae *Macrophthalmus latifrons*
Heloecius cordiformis
Palaemonidae unidentified (1 species)
Tanaidacea *Corophium sp.*

APPENDIX 4B.

Total numbers of macroinvertebrate species collected in shallow subtidal cores at Deceitful Cove and East Arm in the Tamar River estuary, and Squeaking Point and North East Arm in the Port Sorell estuary.

Taxa	Deceitful Cove				East Arm				Squeaking Point				North East Arm			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Foraminifera	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0
Porifera	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Actinaria	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Nemertea *sp.1*	0	3	0	0	0	2	1	0	0	2	0	1	0	0	0	1
Nemertea *sp.2*	0	0	0	0	0	0	0	0	0	0	0	0	3	1	3	4
Nemertea *sp.3*	0	0	0	0	0	1	0	1	1	0	2	0	0	1	0	1
Nemertea *sp.4*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Oligochaeta *sp.1*	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0
Glyceridae *sp.1*	0	0	0	0	0	5	1	2	0	0	1	3	1	0	0	0
Nephtys australiensis	39	42	30	41	32	28	29	28	15	22	17	11	25	22	19	27
Lumbrineris sp.	7	7	5	11	4	1	0	1	10	3	4	5	10	10	19	14
Heteromastus sp.	0	3	1	2	2	1	3	5	3	4	4	8	0	3	0	1
Cirratulidae *sp.1*	0	0	0	0	0	0	0	2	0	0	0	0	1	3	2	1
Magelona sp.	1	5	0	4	3	2	7	2	4	15	4	11	10	12	11	30
Maldane sp.	0	2	0	0	0	3	7	0	3	0	4	5	1	1	2	1
Armandia intermedia	0	0	0	0	0	0	1	0	0	3	3	0	0	0	0	0
Scoloplos simplex	0	0	0	0	0	0	3	0	1	4	0	0	8	3	10	4
Orbinidae *sp.2*	0	1	0	1	3	2	3	2	2	4	4	2	12	3	4	8
Spionidae *sp.1*	0	0	0	0	0	0	1	1	0	0	0	0	1	1	3	0
Terebellides sp.	0	2	1	0	1	2	1	3	9	2	0	3	4	3	1	4
Trichbranchidae *sp.2*	0	0	0	0	0	0	0	1	0	1	0	0	0	1	3	2
Eunicidiae *sp.1*	1	0	1	1	0	0	0	0	0	0	3	0	1	2	1	0
Dorvilleidae *sp.1*	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0
Polynoidae *sp.1*	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Polynoidae *sp.2*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Polynoidae *sp.3*	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Arenicolidae sp.1	0	0	0	0	0	0	0	0	0	1	1	1	3	2	0	2
Neanthes sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Nereidae *sp. 2*	1	0	3	0	0	0	1	0	0	0	2	0	4	4	12	0
Phyllodocidae *sp.1*	0	0	1	0	1	0	0	0	0	0	0	0	2	1	1	2
Eurythoe sp.	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Theora lumbrica	0	0	0	0	0	1	1	3	0	0	0	0	0	1	0	0
Tellina deltoidalis	4	3	5	6	5	7	5	9	3	2	1	4	7	14	7	10
Tellina marginata	0	1	0	0	1	1	1	1	0	0	0	0	0	0	0	0
Tellina botanica	0	0	0	1	0	0	0	0	0	0	0	0	5	1	0	0
Epicodakia tatei	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1
Mysella donacformis	0	1	0	0	2	2	1	2	28	22	16	25	34	31	19	11
Laternula tasmanica	0	2	0	0	0	0	0	0	0	3	0	1	1	1	2	1
Lasaea australis	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	2
Eumarcia fumigata	0	0	0	3	0	0	0	0	1	0	0	0	3	1	2	2
Katelysia peronii	0	0	1	0	0	0	0	0	1	0	0	0	2	1	3	3

Katelysia scalarina	6	18	7	8	1	0	0	1	11	9	10	11	32	62	13	15
Katelysia rhytiphora	1	1	0	4	0	0	0	1	14	11	10	9	3	3	7	0
Xenostrobus inconstans	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Mactra rutescens	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Retusa pelyx	0	1	1	0	0	2	7	1	6	4	1	0	3	3	1	0
Nassarius (Niotha) pauperatus	5	1	2	3	7	13	8	17	6	7	8	8	5	8	5	4
Nassarius (Zeuxis) pyrrhus	5	3	2	6	1	5	0	0	6	8	8	8	5	8	6	4
Pseudoliotia micans	1	0	0	0	1	3	2	0	7	2	1	0	1	0	0	1
Turbonilla (Pyryiseus) fus	0	0	0	0	0	0	0	0	0	0	1	0	1	1	4	2
Bembicium melanostoma	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Littorina (Austrolittorina)	3	1	0	2	0	0	0	1	1	0	1	0	0	4	1	2
Ostracoda sp.1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Gammaropsis sp.	0	1	1	2	0	1	1	0	2	0	0	0	0	0	7	3
Leptochelia dobia	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Birubus sp.	0	0	0	1	0	0	0	0	3	2	1	2	10	8	4	3
Gammaridae sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	3
Gammaridae sp2	0	0	0	1	0	0	1	1	0	0	0	0	3	0	4	3
Corophium sp.	0	0	0	0	1	0	1	0	3	1	0	1	0	0	0	0
Macrophthalmus latifrons	7	3	0	4	4	3	1	3	6	0	1	4	1	2	1	2
Heloecius cordiformis	0	1	2	1	2	3	0	0	1	1	3	2	0	0	0	0
Clycluspis caprella	0	3	0	3	0	0	2	2	0	0	0	0	0	1	3	3
Callianasa ceramica	13	5	7	7	2	0	1	0	2	3	1	1	5	4	2	4
Callianasa sp.2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Mysidacea sp1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Mysidacea sp2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Palaemonidae	0	0	0	0	0	2	0	1	0	0	0	2	0	0	0	0
Paragrapsis gaimardii	3	0	2	0	0	0	0	0	1	3	4	0	0	1	1	2

Persistent Organic Pollutants in Oysters *Crassostrea gigas* and Sand Flathead *Platycephalus bassensis* from Tasmanian Estuarine and Coastal Waters.

J.A. Mondon[1], B.F. Nowak[1] and A. Södergren[2]

[1]*School of Aquaculture, Tasmaian Aquaculture and Fisheries Institute, University of Tasmania, PO. Box 1214, Launceston, Tasmania, Australia 7250*
[2]*Deptment of Ecology, University of Lund, Ecology Building, S-223 62 Lund, Sweden.*

Accepted for publication in *Marine Pollution Bulletin.*

Introduction

Polychlorinated biphenyls (PCBs) have been in use for more than several decades as constituents in a variety of products and in several industrial applications (Bernes, 1998). Owing to their persistent character and lipophilic properties, they are globally distributed. Despite their widespread occurrence in the environment and tendency to accumulate in biota (Harding *et al.* 1997), no information is available on their distribution in marine or estuarine environments of the Tasmanian coastal region. Recent research has shown the presence of PCBs in the fatty tissues of platypus in Tasmania, indicating a widespread distribution of pollutants in the regional freshwater systems, particularly within the Tamar River catchment area (Munday *et al.* 1998).

Polychlorinated biphenyls (PCBs) discharged into aquatic environments adsorb onto particulate matter and are ultimately deposited in bottom sediments (Kalmaz & Kalmaz, 1979) where certain congeners remain available for resuspension for long periods (Swain, 1983). Bioaccumulation of these lipophilic compounds by sediment-dwelling organisms and mobilisation *via* benthic-based food chains may pose ecological hazards (Lester, 1994). Total PCB levels in aquatic organisms are generally related to ambient water concentrations, levels of dietary intake (Kalmaz & Kalmaz, 1979) and most importantly their concentrations within sediments (Shaw & Connell, 1982). Although the potential for accumulation of persistent lipophilic compounds varies with species, size, lipid content and ecological niche of the organism (Nowak, 1997), contamination levels within biota generally reflect the contamination of sediments (Connor, 1984; Mudroch *et al.*1989), with the trophic transfer of PCBs being enhanced by direct contact with contaminated sediment during foraging activity (DiPinto & Coull, 1997).

Exposure to PCBs can elicit a variety of biological and toxicological effects in aquatic organisms including reduction in biomass and growth (Wang *et al.* 1998); endocrine disruption; reproductive impairment and failure; thyroid dysfunction; carcinogenicity; immunodeficiency; neurological disorders; wasting syndrome; and death (for review see Eisler, 1986, and Bernes, 1998).

One area of particular interest in northern Tasmania is Deceitful Cove, situated in the lower reaches of the Tamar River. Over a period of two decades, Deceitful Cove received primary industrial discharge from aluminium and manganese smelting industries and it continues to receive intermittent contamination *via* storm water run-off from these industries and from an adjacent metal recycling plant.

The heavy industrial activity in the area and the potential for partitioning of PCBs in particular from sediments to the biota therefore warrants investigation. A preliminary survey to screen for the presence of organochlorines was undertaken in 1997. Organochlorine levels in sediments, porewaters, benthic fish, and oysters from Deceitful

Cove were compared with those detected from non-industrialised, non-urban sites within the Tamar River, the Port Sorell estuary and the east coast of Tasmania.

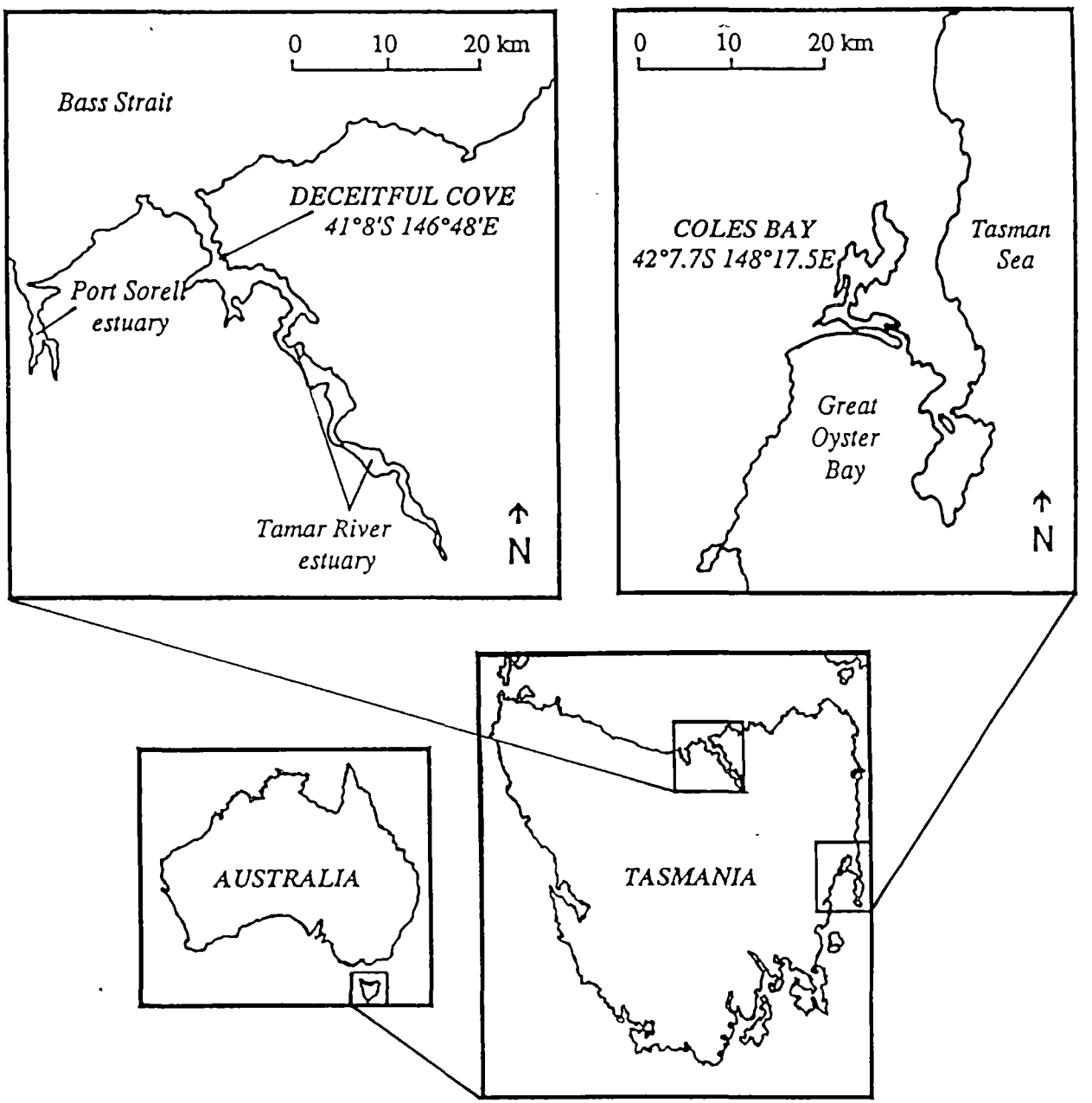


Figure 1. Sampling sites on the Tamar River estuary, Port Sorell estuary and east coast of Tasmania: Deceitful Cove, Deviot, Squeaking Point and Coles Bay.

Methodology

Shallow subtidal sediments and pore waters were extracted from Deceitful Cove in the Tamar River, and also from Squeaking Point in the adjacent Port Sorell estuary, and Coles Bay on the east coast (Figure 1). Whole sediment surface core samples (0-10 cm depth) were collected using acid-washed 7.5 cm diameter benthic core tubes, and were placed in sealed acid-washed Nalgene® polypropelene containers. Vacuum-operated porewater extractors (Winger and Lasier, 1991) were then employed to collect pore water for organochlorine analysis. A fused glass air stone attached with a section of Teflon® tube to a 50ml acid-washed polypropylene syringe was inserted into the extracted pooled sediment cores. Immediately after extraction the sediment and pore water samples were frozen to -20°C until analysis.

Sand flathead *Platycephalus bassensis* (Cuvier 1829) were collected from Coles Bay and Deceitful Cove. Pacific oysters *Crassostrea gigas* were collected from Deceitful Cove and Deviot. Oysters collected by hand from the rocky intertidal zone, and predominantly 3-year old flathead caught by handline (age determined by otolith annual rings), were wrapped in aluminium foil and frozen to -20°C. Subsequently, tissue samples were taken from the dorsal lateral muscle of the fish, and from the adductor muscle of the oysters. Lipids were extracted from both sets of samples by homogenisation with Nanograde acetone/hexane (1:1), using 2 g of homogenised muscle tissue per organism.

The residues in the water were liquid/liquid extracted with hexane, while those in the sediment were retrieved with a mixture of acetone/hexane (1:1). The solvent extracts from water, sediment and lipids were cleaned-up with concentrated sulphuric acid and fractionated on silica gel columns (Bremle & Ewald, 1995).

The samples were analysed for PCBs (30 congeners), α-HCH, β-HCH, γ-HCH, heptachlor, aldrin, heptachlor epoxide, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDT, dieldrin and endrin. The compounds were separated and quantified by gas chromatography (Varian 3500 with EC-detector) with a 60 m DB5 fused capillary column at the Department of

Ecology, Lund University, Sweden. Internal standard PCBs with IUPAC numbers 30, 204 and 209 were used in conjunction with Clophen A60 as an external standard (Ballschmiter & Zell, 1980).

Results and discussion

No organochlorine residues were detected in the porewaters (level of detection for *p,p'*-DDT 0.8pg g^{-1}, for PCB 110 (IUPAC) 0.5pg g^{-1}). With the exception of two cases (SP1 and CB1), the levels of organochlorine compounds present in sediment samples from Squeaking Point and Coles Bay were also below detection levels (Table 1). However, organochlorines were detected in all Deceitful Cove sediment samples, the highest level of PCBs (2681 ng g^{-1} dry weight) occurring in sediments closest to the point where the industrial estate storm water run-off enters the cove.

Table 1. Concentrations of persistent organochlorine compounds in marine sediments from the Tamar River estuary (Deceitful Cove), Port Sorell Estuary (Squeaking Point) and the east coast of Tasmania (Coles Bay).

Location	Site	DW (%)	LOI (%)	Organochlorine concentration (ng g^{-1}) dry weight		
				ΣPCB	ΣDDT	γ-HCH
Deceitful Cove	1*	11.6	18.8	2681	2.2	1.1
	2*	12.9	17.2	734	10.9	1.4
	3*	25.8	12.9	459	7.9	0.5
	4*	19.8	14.9	821	12.8	0.7
	5*					
Squeaking Point	1	73	1.9	n.d	4	3
	2	70	3.5	n.d.	n.d	n.d
	3	60	4	n.d.	n.d.	n.d
	4	62	3.9	n.d.	n.d.	n.d
	5	63	4.2	n.d.	n.d.	n.d
Coles Bay	1	74	2.7	5	n.d.	n.d
	2	85	0.8	n.d.	n.d.	n.d
	3	80	0.4	n.d.	n.d.	n.d
	4	84	0.5	n.d.	n.d.	n.d
	5	78	0.5	n.d.	n.d.	n.d

* denotes 4 pooled samples
DW (%) = dry weight
LOI (%) = loss on ignition

Sand flathead from Coles Bay were clear of organochlorine residues except for 42 ng g^{-1} lipid weight of lindane in one fish. The level of PCBs in Deceitful Cove sand flathead ranged from 667ng g^{-1} lipid weight to 4899 ng g^{-1}, with only two fish exhibiting concentrations below 1000 ng g^{-1} (Table 2). The levels of ∑DDT detected in these fish ranged from 10 to 243 ng g^{-1} lipid weight, with *p,p'*-DDE the primary contributing residue in all samples. Five of the ten Deceitful Cove sand flathead exhibited γ-HCH levels ranging from 8 to 28 ng g^{-1} lipid weight.

Table 2. Concentrations of persistent organochlorine compounds in sand flathead *Platycephalus bassensis* from the Tamar River (Deceitful Cove) and Coles Bay region in Tasmania.

Location	Sample	% Lipid	Organochlorine residues (ng g^{-1}) lipid weight					
			∑PCB	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	∑DDT	γ-HCH
Coles Bay	1	0.02	n.d	n.d	n.d	n.d	n.d	n.d
	2	0.01	n.d	n.d	n.d	n.d	n.d	n.d
	3	0.02	n.d	n.d	n.d	n.d	n.d	n.d
	4	0.01	n.d	n.d	n.d	n.d	n.d	n.d
	5	0.01	n.d	n.d	n.d	n.d	n.d	n.d
Deceitful Cove	1	0.8	1069	105	23	n.d	129	12
	2	0.8	1438	10	n.d	n.d	10	n.d
	3	0.6	1154	145	n.d	n.d	145	13
	4	0.9	1242	74	n.d	n.d	74	n.d
	5	0.9	1005	37	n.d	n.d	37	n.d
	6	0.8	1829	176	39	28	243	8
	7	0.7	667	123	23	n.d	146	n.d
	8	0.8	4899	33	n.d	n.d	33	28
	9	0.9	1387	164	24	15	204	n.d
	10	0.7	826	107	16	n.d	123	16

% Lipid = Percentage of extractable lipid from the lateral muscle of the finfish

The PCB levels in oysters from Deceitful Cove were not significantly different from those collected at Deviot. The PCB concentrations in both sets of samples were high, ranging from 1499 to 8339 ng g^{-1} and from 2644 to 8836 ng g^{-1} lipid weight, respectively. However, DDT levels in oysters from Deviot were significantly higher ($p=0.0119$) than those recorded at Deceitful Cove, the difference averaging 4-fold.

Table 3. Concentrations of persistent organochlorine compounds in the Pacific oyster *Cassostrea gigas* from the Tamar River estuary.

Location	Sample	% Lipid	Organochlorine residues (ng g^{-1}) lipid weight					
			ΣPCB	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	ΣDDT	γ-HCH
Deceitful	1	0.9	1911	0.7	1.4	n.d	2.1	0.3
Cove	2	1	1499	44	41	n.d	85	n.d
	3	0.6	8339	34	n.d	n.d	34	34
	4	0.3	7838	176	n.d	n.d	176	69
	5	0.5	8034	43	n.d	n.d	43	n.d
Deviot	1	0.4	5095	197	n.d	n.d	197	94
	2	0.3	8836	261	n.d	n.d	261	232
	3	0.4	3942	483	n.d	n.d	483	59
	4	0.5	2644	227	77	n.d	304	62
	5	0.4	5181	148	n.d	n.d	148	99

% Lipid = Percentage of extractable lipid from the abductor muscle of the oyster.

Polychlorinated biphenyls in Deceitful Cove oysters were present at almost twice concentration detected in fish from the same location. Conversely, DDT levels in the same Deceitful Cove oysters amounted to only 60% of the levels detected in fish. Heptachlor, heptachlor epoxide and α-HCH were not detected in sediment, sand flathead or oysters.

The results indicate the presence of PCB and DDT contamination in Deceitful Cove sediments and biota, whereas the Port Sorell and Coles Bay samples were relatively clean. Furthermore, these data tentatively support the tenet of Munday *et al.* (1998) of widespread contamination of the Tamar River catchment area.

Evidence from oysters suggests contamination in the Tamar River estuary may not be limited to the lower estuary waters in close proximity to the heavy metal smelting and recycling operations. It appears that potentially high levels of organochlorines may extend to at least the middle reaches of the Tamar River. However, considering the small sample size and the absence of spatial data above and below both sites, the results quoted here may be indicative of either general organochlorine contamination along the length of the estuary, or could reflect the presence of two hot spots within the estuary.

Despite the gradual phasing-out of DDT in Tasmania from 1973 - 1987, non-metabolized DDT was present in two fish from Deceitful Cove, indicating the possibility of relatively recent illegal use or disposal. Although the use of lindane has been highly restricted in Tasmania, its presence in some of the oysters and flathead sampled in the Tamar River is not unexpected, pointing to very slow metabolism of these compounds within the ecosystem.

The work of Shaw and Connell (1982) in the Brisbane River estuary in Queensland noted that sea birds such as silver gulls (*Larus novahollandiae*) and pelicans (*Pelecanus conspicillatus*) contained higher levels of PCBs than other estuarine organisms, and the level of PCB accumulated was dependant on the PCB content of food and the food quantity consumed. Whilst PCB levels in the Brisbane River estuary flathead exceeded those in the Tamar by 10- to 20-fold, there is a possibility that higher trophic organisms of the Tamar River estuary could exhibit similar levels to those from the Brisbane River. PCB levels detected in sand flathead (*Platycephalus bassensis*) in Port Phillip Bay (Nicholson *et al.*, 1994) suggest a similar degree of environmental contamination to that of Deceitful Cove. However, the areas of urbanisation and industrialisation around both Port Phillip Bay and the Brisbane River are markedly larger than Deceitful Cove.

The suggestion that biota may constitute an important sink for the higher-chlorinated congeners is particularly relevant for the Tamar River, as higher-chlorinated PCBs exhibit greater levels of bioaccumulation in fish than the lower-chlorinated congeners (Vanbavel *et al.*, 1996). Furthermore, the specific association of highly-chlorinated congeners with the silt fraction of sediments (< 63 μm; see Pierard *et al.*, 1996) infers that in the Tamar River estuary, where sediments constitute mostly fine mud from the middle to upper reaches of the river (Foster, *et al.*, 1986), significant contamination of biota is highly probable.

These findings are significant in terms of the long-term health implications for users of the Tamar River marine and estuarine ecosystem. The lower estuary is popular for recreational

fishing, particularly within Deceitful Cove, and oysters are routinely collected at a number of locations in the lower reaches of the Tamar (Pirzl & Coughanowr, 1997). Marine fish culture is also conducted within this region. Deceitful Cove and the adjacent waters of Port Dalrymple support a wide variety of pelagic and benthic fish species, shore birds, sea eagles, and occasionally seals, penguins and dolphins. The incidence of larger cetaceans visiting the estuary during their annual migration to the Southern Ocean has also increased over the last decade.

The consumption of oysters from the Tamar River is not common, due to an extensive public awareness campaign warning of excessive heavy metal accumulation. However, the potential exists for the accumulation of PCBs by humans *via* the consumption of flathead and other common non-migratory finfish species such as greenback flounder and cod. The sand flathead caught in shallow water (approx. 10 m) at Deceitful Cove were young, with relatively low body fat. Fish with a higher lipid content such as cod, or larger sand flathead from deeper sites in the Tamar estuary may well exhibit higher levels of contamination (Nicholson *et al.*, 1994). Once accumulated, the reduction in PCB levels is slow for humans and other biota with long life-spans (Harrad *et al.*, 1994). Even though the potential cancer risk to humans from ingesting PCBs in fish and shellfish may be reduced by environmental degradation (Barron *et al.*, 1994), not all PCBs are metabolisable (Looser & Ballschmiter, 1998). It is also possible that the fish themselves may be adversely affected by these compounds, particularly as PCBs are transferred from females to their offspring (Harding *et al.*, 1997).

In all cases the Deceitful Cove sediment samples exceeded the Sediment Quality Guidelines for polychlorinated biphenyls, these involving an ERM (effects range median) concentration of 180 $\mu g\ kg^{-1}$ dry weightt (Long *et al.*, 1995). This indicates that toxicity from PCBs is likely to occur at some level within the aquatic community. However, the long term effects of organochlorine contamination at either the individual, population, community or ecosystem level in the Tamar River are unknown.

The results from this survey are significant with respect to the long-term health of marine biota and humans exposed to organochlorines in sediments and through dietary intake in the Port Dalrymple - Deceitful Cove region. Further research is warranted to evaluate the extent of contamination along the Tamar River estuary to identify the scale of potential impacts from persistent organic pollutants.

References

Ballschmiter, K. & Zell, M. (1980). Analysis of polychlorinated biphenyls (PCBs) by glass capillary gas chromatography. Composition of technical Aroclor and Clophen PCBs mixtures. *Fresenius Zeitschrift fuer Analytische Chemie, 302*, 20-31.

Barron, M.G., Yurk, J.J. & Crothers, D.B. (1994). Assessment of potential cancer risk from consumption of PCBs bioaccumulated in fish and shellfish. *Environmental Health Perspectives, 102*, 562-567.

Bernes, C. (1998). *Persistent Organic Pollutants* (Monitor 16): Swedish Environmental Protection Agency, Solna, Sweden.

Bremle, G. & Ewald, G. (1995). Bioconcentration of polychlorinated biphenyls (PCBs) in chironomid larvae, oligochaete worms and fish from contaminated lake sediment. *Marine and Freshwater Research, 46*, 267-273.

Connor, M.S. (1984). Comment on fish / sediment concentration ratios for organic compounds. *Environmental Science and Technology, 19*, 199.

DiPinto, L.M. & Coull, B.C. (1997). Trophic transfer of sediment-associated polychlorinated biphenyls from meiobenthos to bottom-feeding fish. *Environmental Toxicology and Chemistry, 16*, 2568-2575.

Eisler, R. (1986). *Polychlorinated Biphenyl Hazards in Fish, Wildlife, and Invertebrates: A Synoptic Review.* (Contaminant hazard reviews 7): Fish and Wildlife Service,U.S. Department of the Interior, Washington.

Foster, D.N., Nittim, R. & Walker, J. (1986). *Tamar River Siltation Study* (Water Research Laboratory Technical report 85/07): University of New South Wales, Sydney.

Harding, G.C., Leblanc, R.J., Vass, W.P., Addison, R.F., Hargrave, B.T., Pearre, S., Dupuis, A. & Brodie, P.F. (1997). Bioaccumulation of polychlorinated biphenyls (PCBs) in the marine pelagic food web, based on a seasonal study in the Southern Gulf of St. Lawrence, 1976-1977. *Marine Chemistry, 56*, 145-179.

Harrad, S.J., Sewart, A.P., Alcock, R., Boumphrey, R., Burnett, V., Duartedavidson, R., Halsall, C., Sanders, G., Waterhouse, K., Wild, S.R. & Juones, K.C. (1994). Polychlorinated biphenyls (PCBs) in the British environment - Sinks, sources and temporal trends. *Environmental Pollution, 85*, 131-146.

Kalmaz, E.V. & Kalmaz, G.D. (1979). Transport, distribution and toxic effects of polychlorinated biphenyls on ecosystems: review. *Ecological Modellin, 6*, 223-251.

Lester, D.C. & McIntosh, A. (1994). Accumulation of polychlorinated biphenyl congeners from Lake Champlain sediments. *Environmental Toxicology and Chemistry, 13*, 1825-1841.

Long, E. R., MacDonald, D. D., Smith, S. L. & Calder, F. D. (1995). Incidence of adverse effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management, 19*, 81-97.

Looser, R. & Ballschmiter, K. (1998). Does the deep-sea function as the ultimate sink for semivolatile POPs? Paper presented at the SETAC-Europe, Bordeaux.

Mudroch, A., Onuska, F.I. & Kalas, L. (1989). Distribution of polychlorinated biphenyls in water, sediments, and biota of two harbours. *Chemosphere, 18*, 2141-2154.

Munday, B.L., Stewart, N.J. & Sodergren, A. (1998). Occurrence of polychlorinated biphenyls and organochlorine pesticides in platypuses (*Ornithorhynchus anatinus*) in Tasmania. *Australian Veterinary Journal, 76*, 129-130.

Nicholson, G.J., Theodoropoulos, T. & Fabris, G.J. (1994). Hydrocarbons, pesticides, PCB and PAH in Port Phillip Bay (Victoria). *Marine Pollution Bulletin, 28*, 115-120.

Nowak, B.F. (1997). Biological significance of xenobiotic residues in fish: review. *Toxicological and Environmental News and Reviews, 4*, 148-154.

Pierard, C., Budzinski, H. & Garrigues, P. (1996). Grain-size distribution of Polychorobiphenyls in coastal sediments. *Environmental Science and Technology, **30**,* 2776-2783.

Pirzl, H. & Coughanowr, C. (1997). *State of the Tamar Estuary* (Supervising Scientist Report 129). Commonwealth Department of Environment , Sport and Territories, Canberra.

Shaw, G.S. & Connell, D.W. (1982). Factors influencing concentrations of polychlorinated biphenyls in organisms from an estuarine ecosystem. *Australian Journal of Marine and Freshwater Research, 33*, 1057-1070.

Swain, W.R. (1983). An overview of the scientific basis for concern with polychlorinated biphenyls in the Great Lakes. In *PCBs: Human and Environmental Hazards* (D'Itri, F.M. and Kamrin, M.A., eds.), pp. 11-48. Butterworth Publishers, Woburn.

Vanbavel, B., Andersson, P., Wingfors, H., Ahgren, J., Bergqvist, P.A., Norrgren, L., Rappe, C. & Tysklind, M. (1996). Multivariate modeling of PCB bioaccumulation in three-spined stickleback (*Gasterosteus aculeatus*). *Environmental Toxicology and Chemistry, 15*, 947-954.

Wang, J.S., Chou, H.N., Fan, J.J. & Chen, C.M. (1998). Uptake and transfer of high PCB concentrations from phytoplankton to aquatic biota. *Chemospere, 36*, 1201-1210.

Winger, P.V. & Lasier, P.J. (1991). A vacuum-operated pore water extractor for estuarine and freshwater sediments. *Archives of Environmental Contamination and Toxicology, 21*, 321-324.